DEC 26 1923

VOLUME 13 NUMBER 12

DECEMBER, 1923

Phytopathology

Official Organ of the

American Phytopathological Society

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PUBLISHED MONTHLY BY THE SOCIETY

OFFICE OF PUBLICATION

8 WEST KING STREET, LANCASTER, PA.

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PHYTOPATHOLOGY

VOLUME XIII NUMBER 12

DECEMBER, 1923

NOTES ON THE FAILURE OF GRAPEVINES TO SET FRUIT AND ON SHELLING

ARTHUR S. RHOADS

WITH PLATE XXI

The failure of grapevines to set fruit, or to retain their fruit after it has developed more or less fully, is a trouble of considerable economic importance. Both this non-setting of grapes and their falling off after they have developed more or less fully is termed "coulure" by the French viticulturists.

While the failure of the flowers to set fruit, or of the berries to develop appreciably, has long been recognized by European viticulturists, and some phases of it by American horticulturists engaged in grape-breeding experiments, this trouble has been given but scant attention by American pathologists. From a consideration of the American pathological literature the impression is gained that the non-setting of grapes is of but little importance in comparison to the falling of the more or less fully developed fruit, or "shelling" or "rattles," as this trouble is commonly designated in this country.

Observations by the writer indicate that the non-setting of grapes is of far greater importance as a factor of loss than the commonly mentioned shelling. The undue prominence which shelling has attained in comparison to non-setting is largely due to the much greater conspicuousness of the loss caused by the former, the falling of some of the well-developed berries attracting the attention of even the most casual observer, whereas large numbers of the flower clusters may fail to set fruit or even blight and drop off entirely within a few days and entirely escape the casual observer or excite but little comment as a rule.

In California, Bioletti (1) has applied the term "early coulure" to the failure of the flowers to set fruit or the dropping of the berries without developing appreciably, and the term "late coulure" to the falling or shelling of the berries after they are well developed. Bioletti's distinctions seem very appropriate and should prove useful, especially in the absence of any other term, for the earlier form of this grape trouble.

The non-setting of the grapes, or early coulure, if we accept the more convenient term, has been investigated by a large number of observers and a variety of causes assigned. These may be either constitutional or accidental.

Under early coulure as a result of constitutional causes by far the most important factor to be considered is the lack of self-fertility of certain varieties due to imperfect development of the flowers or to lack of functional activity of the pollen produced. The work of a number of investigators has shown that a large number of grape varieties are more or less incapable of setting fruit if planted alone, but that, like the pistillate strawberries, they must depend upon the pollen of other varieties for perfect fertilization and fruitfulness. In other instances, flowers may be seemingly perfect in their organs and yet fail to develop fruit. Müller-Thurgau (6) regards this as an inheritable character of certain vines, the only remedy for which appears to be ringing the vines, which tends to lessen the amount of water supplied and to concentrate the liquid nourishment carried to the flowers.

Early coulure as a result of accidental causes has been attributed to such factors as unfavorable weather conditions or fungous attacks occurring at the flowering time, spraying while in bloom, improper pruning, a deficiency in the supply of reserve materials in the canes due to its depletion by excessive vegetative growth or by overbearing the preceding year, unsuitable or exhausted soil, and the absorption of excessive material from soils extremely poor in nitrogen and phosphoric acid. The writer wishes to record observations on the first two of these factors.

In 1922 all the grapevines in the vineyard of the Missouri State Fruit Experiment Station that had come into full bearing, produced a very satisfactory crop of fruit and in the spring of 1923 the same vines produced an abundance of blossom clusters and had every indication of bearing an equally good crop. However, the weather conditions that prevailed during the blooming period of the grapes resulted in a more or less severe reduction of the fruit upon certain varieties. This was occasioned largely by the failure of the fruit to set but was aided somewhat in a few varieties by the early development of black rot, which attacked the flower clusters even before the blossoms opened.

The observations made upon the station vineyard, at least those in so far as the counts were concerned, were confined to that portion of the vineyard in full bearing, which consisted of several different varieties in their fifth year. The Champion, which is the earliest bloom-

ing variety, was in full bloom on May 30, and the Carman, which is a very late blooming variety, had passed the climax of its bloom on June 18.

The fruit loss due to failure to set as a result of the prevalence of rain during the blooming period, estimated after the set of the remainder had been assured and checked up later in the season after the berries had become about half grown, was as follows:

Variety	Period of Blooming	Per cent Loss
Champion	Early	Less than 1
Cottage	"	2
Barry	Midseason	Less than 1
Concord	"	Less than 1
Daisy	"	4
Eclipse	"	25
Hartford	"	4
Herbert	"	5
Hernito	"	20
Hicks	"	2
King	"	4
Moore's Early	"	50
President	"	5
Captain	Late	20
Cloeta	"	1
Fern	"	50
Lomanto	"	5
Muench	"	80
Carman	Very Late	35

The unfertilized berries either fell off the flower clusters before attaining any appreciable development or else developed very tardily as compared to normal berries, and then fell off while still very small (Pl. XXI fig. 1). This same trouble was noted also in other small grape plantings at Mountain Grove and in vineyards in other sections of Missouri.

In four varieties the estimation of the fruit loss was complicated by the occurrence of blossom blight caused by the black-rot fungus, *Guignardia bidwellii* (Ell.) V. & R. The flower clusters attacked by this fungus quickly became brown, soft and rotten, the lower end being the first to become infected as a rule (Pl. XXI fig. 2). Within a few days the entire flower cluster became involved and dried up and dropped off, the scars left by the abscissed peduncles being the only indication that the shoots had developed flower clusters. Numerous pustules of the black-rot fungus appeared in the bases of more or less of the new growth of the four varieties infected and, in a few cases, occurred even on the peduncles of the flower clusters. In addition to the figures already given for fruit

losses as a result of the prevalence of rain during the blooming period it was estimated that there was a further loss of 5 per cent of the potential crop of the Moore's Early, 50 per cent of that of the Hernito, 75 per cent of that of the Captain, and 35 per cent of that of the Carman due to black-rot alone.

An inspection also was made of three species of wild grapevines which occur abundantly on land adjoining the station. Of these species, which were in full bloom on June 20, blossom blight caused by black-rot was abundant on *Vitis aestivalis* Michx. and *V. cordifolia* Michx., practically all the flower clusters on some vines rotting back from the tip, and later turning brown, shrivelling and falling off. No evidence of black-rot was to be observed on any part of the vines of *V. cinerea* Engelm., even though growing in association with heavily infected vines of the other two species, this species appearing to be quite immune.

The meteorological records taken at the station, which are given below for the blooming period of the grapes, show that cool, rainy weather prevailed throughout the blooming period.

Date	Temperature		Precipitation in inches	General character of day
	Maximum	Minimum		
May 28	78	58	—	Partly cloudy
29	81	62	—	Clear
30	83	60	.09	Partly cloudy
31	85	62	trace	" "
June 1	86	65	trace	Clear
2	79	61	—	Partly cloudy
3	83	62	1.04	" "
4	79	64	.58	Cloudy
5	82	63	—	Partly cloudy
6	81	62	—	" "
7	83	61	.28	" "
8	83	60	—	" "
9	80	62	—	Clear
10	73	63	.67	Cloudy
11	75	64	.47	"
12	77	57	.18	"
13	69	53	trace	"
14	79	59	—	Clear
15	77	63	—	Partly cloudy
16	86	65	.08	" "
17	84	77	.46	" "
18	85	66	.03	" "
19	86	67	—	" "
20	84	67	—	Clear

Hedrick (3, p. 77) concludes that rain and the cold and wind that usually accompany it at blossoming time cause the loss of more fruit in New York than any other climatal agencies. The damage is done in several ways. In addition to the most obvious injury, namely, the washing of the pollen from the anthers, the secretion of the stigmas, which is so essential to the proper germination of the pollen, also is washed away or becomes so diluted that the pollen does not germinate. It is further probable that the chill of the rainy weather decreases the vitality of the pollen. Hedrick (4, p. 368) also states that further investigations at the Geneva Station have shown that the pollen grains of practically all fruits burst and will not germinate in water. From this work he is of the opinion that there can be little doubt but that the action of rain on pollen is quite as potent in preventing a set of fruit as is its injurious effect on the stigmatic fluid. Casella (2) states that in the grape, rain was found to cause the cap or calyptrum to adhere to the stigma and thus prevent pollination.

Quite as important as any of the above considerations, and possibly more so, is the fact that rain prevents the distribution of pollen by the wind and must effectually prevent bees and insects from carrying it. Weather conditions involving rain and a humid atmosphere also have much to do with the development of parasitic fungi, which may quickly destroy the blossoms or even cause a blight of whole blossom clusters. This, however, is an entirely different condition than the dropping of the blossoms as a result of incomplete fertilization.

Low temperatures and rainy weather during the blooming period may be a factor in reducing the set of the fruit in still another way. According to the investigations of Müller-Thurgau (5) on the falling of grape flowers, dull, cool weather during blossoming is especially favorable for the growth of leafy shoots which aid in the depletion of the reserve food materials that otherwise would be available for the development of the inflorescence, so that the nutrition is not sufficient for the blossoms. He states, further, however, that such a starving of the blossom cluster and, consequently, a more or less extensive shelling of the blossoms will occur also with weather favorable for blooming if abundant nitrogen is present in the soil, or if virgin soil is used for the cultivation of grapes when the luxuriant development of the vegetative organs limits the further development of the blossoms.

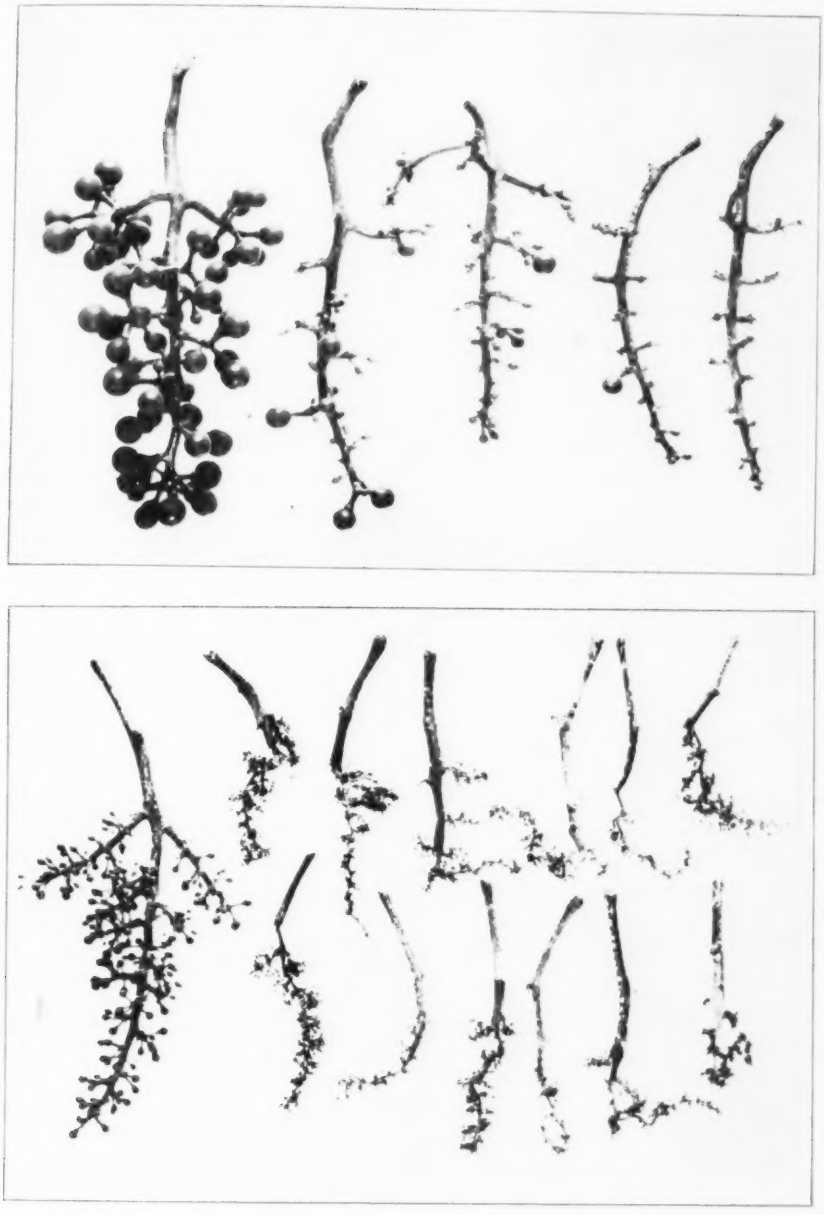
Subsequent investigations by Müller-Thurgau (6) indicate that not only long-continued cold, rainy weather, but a long period of low temperature alone, or excessive dry weather have been found conducive to

the failure of the fruit to set. It is thought by him that low temperature exercises an unfavorable influence on the metabolism of the flowers and that evaporation and the formation of sugar in the leaves are retarded, as is likewise the solution and utilization of the organic reserve material.

Shelling of the more or less fully developed berries, or late coulure, also is due to a variety of causes which come under the category of unbalanced nutrition and unfavorable climatic conditions. The agencies that contribute to shelling from these causes generally are considered to be the following: a weakening of the vines due to over-bearing, heavy vegetative growth in proportion to the root system, attack of the vine by root-rot, or to such factors as excessive nitrogen supply emphasized by over-tillage and prolonged drought or excessive rains followed by drought. It has also been held that a lack of potash is in many cases the primary cause, although there is considerable evidence that this is not a factor.

Grapes affected with shelling from purely physiological derangements engendered by these causes usually fall two or three weeks before maturity, the normally fibrous, connecting tissue of the pedicel cleaving smoothly at the point of junction with the berry. The berries at the end or at the extremity of the shoulder of the cluster, as a rule, are the first to fall. The affected berries, particularly of the green varieties, often exhibit a peculiar, though indistinct, mottling of the surface; the skin becomes abnormally thick and the whole berry is harder than normal berries of the same age. The interior of such a berry shows a brown zone immediately beneath the skin and the taste is noticeably insipid as compared to the tart, astringent flavor of the normal, unripe berries.

The shelling of mature or nearly mature grapes may also occur as the result of certain fungous diseases of the fruit, although these have been denied as the primary cause of this trouble as understood in American literature. This has been due to the fact that the term shelling has been limited hitherto to the dropping of the fruit as a result of purely physiological disorders. A typical shelling of the fruit, however, occurs when the berries are attacked by such fungi as the brown-rot form of downy mildew (*Plasmopara viticola* (B. & C.) Berl. & De Toni), the dead-arm disease (*Cryptosphaerella viticola* (Red.) Shear), the bitter-rot (*Melanconium fuligineum* (Scrib. & Viala) Cav.), the white-rot (*Coniothyrium diplodiella* (Speg.) Sacc.), and the ripe-rot (*Glomerella cingulata* (Atk.) Spauld. & v. Schrenk). Even in the case of shelling caused by these fungous diseases, the fungous disease is not always evident. During the course of the writer's investigations of grape diseases during the



NON-SETTING AND BLACK ROT BLIGHT OF GRAPE FRUITS

FIG. 1. (Upper.) Failure of Moore's Early grapes to set fruit as a result of the cold, rainy weather that prevailed during the blooming period, showing a normal cluster at the left. $\frac{5}{8}$ natural size.

FIG. 2. (Lower.) Blight of Carman grape blossom clusters caused by black-rot, showing normal cluster at the left. $\frac{1}{2}$ natural size.

summers of 1922 and 1923 it was noted in vineyards at Neosho and other points in Missouri, where the grapes were attacked by bitter-rot, that many of the ripe grapes would shell off during the handling incident to picking and packing. Most of these, it is true, showed more or less conspicuous pustules, while others appeared perfectly normal to the eye, although they were infected and developed pustules after being placed in a moist chamber for a few hours or sometimes days.

Inasmuch as shelling of grapes may be caused by certain fungous diseases alone, it is evident that our usage of this term will have to be enlarged beyond the scope of miscellaneous physiological disorders, to which it has been limited previously.

MISSOURI STATE FRUIT EXPERIMENT STATION,
MOUNTAIN GROVE, MISSOURI.

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A DISEASE OF TOMATO CAUSED BY PHYTOPHTHORA MEXICANA, SP. NOV.

J. W. Hotson and Lena Hartge

With Plates XXII and XXIII and One Figure in the Text

INTRODUCTION

The genus Phytophthora was first named and described in 1876 by Anton de Bary (1), who was at that time making an extensive study of the disease producing potato blight. To the organism causing that disease he gave the name *Phytophthora infestans*, by which name it has since been known. Saccardo lists eight species in his Sylloge Fungorum (12). There are now more than twenty known species, four of which have been reported as attacking tomato. These four are as follows: tomato blight, caused by *P. infestans* (Mont.) de Bary (9); buckeye rot of tomato fruit, caused by *P. terrestria* Sherb. (13); collar rot, caused by *P. cryptogea* Pethy. (7), and the fourth, a damping off, stem-girdling Phytophthora as yet undescribed and unnamed (8). The present paper has to do with a fifth disease, caused, it is believed, by another species of Phytophthora which has been under observation and culture for more than three years. The organism herein described was isolated from some tomatoes shipped into Seattle, Washington, from Mexico in the early summer of 1917. Transfers were made of this culture from time to time to keep it alive until 1920, at which time the cultural study was begun.

The fungi of the genus Phytophthora were for a long time thought to be obligate parasites, but in 1900 two Frenchmen, Matruchot and Molliard, succeeded in growing *P. infestans* on artificial media. Five years later, Clinton, working at Connecticut Agricultural Experiment Station, reported somewhat similar results (2). Jones and Giddings (3), of the University of Vermont in 1909, also carried on some research work along this line with varied success. Since that time considerable cultural work has been done on this genus.

THE MYCELIUM

The mycelium of the fungus under consideration grows readily on artificial media, producing more or less dense growth on the surface of some and growing quite deeply into the substratum of others. Within twenty-four hours radiating strands of mycelium one-half inch or more long may be seen growing out from the point of inoculation. As the growth increases, if the growth be aerial in character, the medium

becomes covered with an even, white coat of mycelium, and on the other hand, if the growth be substratal in character, the natural color of the medium prevails, due to the sparse surface growth. The former is true of its growth on the lima bean agar, and the latter is characteristic of its growth on the oat agar, while on the tomato media there seems to be both aerial and deep substratal growth.

The hypha averages 7.7 μ in diameter, and is profusely branched and non-septate. In its younger stages it is full of dense, granular protoplasm, interspersed with numerous oil globules. As has been observed by other workers on the genus Phytophthora, the tip of the branches often burst when mounted in water and the contents ooze out in oily globules. The branching of the mycelium is very irregular. Some of the branches are of a short, knob-like character, sometimes touching and anastomosing. Where the mycelium penetrates the medium it gives the peculiar knobby appearance shown in Plate XXII, figs. 2 and 3. The knobby appearance is not a new feature, as it has been previously mentioned in the description of other species. Reddick (8), for instance, found swellings and distortions of the hyphae of a Phytophthora grown on beef bouillon, or beef agar with sugar. In the species under consideration, however, liquid media seem to give straight, long, slender filaments with but little of the irregularity that is characteristic of its growth on solid media. In older cultures the mycelium becomes septate, a condition noted by other workers on other species of Phytophthora. At this stage the filaments are usually empty, or if they contain protoplasm, it seems to have lost its continuity and to be disintegrating.

The tomato fruit, when inoculated, shows the mycelium growing in all parts of it. It seems to be intercellular in its character, appearing on the surface after about five days and within two weeks covering the entire surface with a white growth. In the stem of the tomato plant the hyphae are usually found to be intercellular, rarely intracellular, and occasionally penetrating the spiral vessels and growing up through the vascular system (Plate XXIII, fig. 15). In the leaf, roughly mounted, the mycelium may be seen through the epidermal tissue ramifying in all directions between the cells.

CONIDIOPHORES

The conidiophores (Plate XXII, fig. 11) are found in varying numbers on all cultural media, profuse on tomato agar, but rather scattering and scanty on oat agar. At different times of the year they vary greatly in abundance. In the spring and summer months they appear in great

numbers. During the past winter when conidia were desired for inoculation purposes, it was observed that they were produced very sparingly both on old and on freshly made media of various kinds. In many cases none at all were found. But with the advent of spring, conidia came again on all cultures in great numbers. This probably was a temperature condition, as the past winter was an exceptionally cold one for Seattle, and no similar conidial conditions were observed in previous years.

Cultures were made in Van Tieghem cells and the development of conidia watched. The conidiophore (Plate XXII, figs. 1, 4, 5, 6) appears first as a simple stout branch at the apex of which a single conidium is borne. The branch at the base of the conidium swells slightly, the conidium is pushed aside, and the branch elongates, producing another apical conidium. Simultaneously with this growth the primary conidium is pushed out on a long slender stalk arising by growth at its base. Often the conidia are produced so close together and so abundantly that the conidiophore assumes a cymose appearance. Rarely does the conidiophore have the swollen knob just below the branch as in *P. infestans*. But this feature is neither a common nor a typical characteristic of this species.

The conidiophores on the leaves of inoculated tomato plants may be seen pushing out through the stomata, the base always bulging noticeably just after reaching the surface of the leaf (Plate XXII, fig. 10).

THE CONIDIA

The conidia begin to form in forty-eight hours after new cultures have been made and begin to germinate immediately on reaching maturity They vary greatly in size, ranging from 16 to 77 μ in length and from 16 to 33 μ in width. Those coming from the tomato fruit and from tomato plant are of an elongated type, often measuring twice as long as wide (44 $\times$ 22 μ to 46.2 $\times$ 24.2 μ). In the early stages of development they are filled with densely granular protoplasm surrounding a large vacuole. When the conidia reach maturity the vacuoles seem to have partially disappeared. There is also a great variety of forms, some having length and width nearly equal and others being twice as long as wide, while others are quite irregular in outline (Plate XXIII, fig. 10). All of them have exaggerated papillae through which the zoospores escape on germination of the conidia.

On several occasions the germination from beginning to its conclusion was watched. The following account is the result of observations of the

growth of the fungus in Van Tieghem cells. The first evidence of germination seemed to be the disappearance of the vacuole and slight, hardly noticeable demarcations, as if the protoplasm were dividing, and then the whole contents of the cell began to move, going about in a circle. A space appeared between the moving ball and the wall of the conidium. In this space one could discern later wave-like motions as if cilia were moving. Definite bodies could be seen rounding up and occasionally one extended farther out than the others into the space next to the cell wall (Plate XXIII, fig. 17). The motion continued for more than one hour with short intervals of rest. Then suddenly the zoospores thus formed seemed to extricate themselves from each other and darted through the papilla-like projection into the outer liquid (Plate XXII, fig. 7). This emptying process occupied only a few seconds. In no case were the zoospores seen to be liberated into a vesicle or bladder as mentioned and pictured by Rosenbaum for *P. arecae* (10).

The zoospores are oval, elongated with a slight depression on one side. No cilia were visible except in the wave-like motion when the zoospores were moving. They soon settle and round up, germinating immediately by a thick germ tube with a diameter almost equal to that of the spore itself.

Occasionally only part of the zoospores escape from the zoosporangium, the remaining ones germinating in the interior and sending out their germ tubes through the sporangial wall in different directions (Plate XXII, fig. 9). Under some conditions there seems to be little evidence of the rounding up of zoospores, but a number of germ tubes are pushed out through the papilla end of the conidium (Plate XXII, fig. 8). At other times a conidium germinates by sending out a single germ tube, often with a single conidium forming at its end (Plate XXIII, figs. 7 and 21). This conidium may germinate by a germ tube which, in turn, forms another conidium at the tip, so that as many as four successive conidia have been observed to form and germinate in a series, the last one being in a young stage full of dense protoplasm. These conidia after they have germinated being somewhat rounded and intercalary, resemble chlamydospores, but they have a distinct papilla as well as the general shape of conidia when they are formed.

CHLAMYDOSPORES

Chlamydospores, asexual intercalary spores, are sparingly produced. Only during one summer did they seem to be produced in numbers. They are of the same general appearance when mature as conidia, with

dense, granular protoplasm with many oil globules and one or two vacuoles visible. When old they become slightly yellow. On germination each produces a single germ tube, often with a conidium produced at the end of a short hypha (Plate XXIII, fig. 16). The chlamydospores are almost spherical in shape, ranging from 28 to 44 μ in diameter. The kind of media does not seem to be a factor on their production as they were found occasionally on all media used.

OOSPORES

Oospores have been found fully developed on cultures eight days old during the colder weather. Rather longer time seems to be necessary when the mycelium is producing conidia readily or when the temperature is higher. Under these conditions four weeks may elapse before the formation of oospores. At first they are about the color of the conidia, with the same granular protoplasmic contents, but without the vacuole (Plate XXIII, figs. 3, 4, 14). As they mature they become a deep yellow with a thick, rather smooth wall, which gives the appearance of having from two to three layers (Plate XXIII, figs. 2, 6, 8, 11, 13). At a still later stage the contents are often broken into larger particles. In general they are the same type of oospore as is found in *P. phaseoli*, with the antheridial cell at the base. They are nearly spherical and measure from 24 to 37.4 μ in diameter with a wall 4.4 μ thick.

The antheridium, in its first appearance, is a swollen portion of the filament and is ovoid in shape. In older cultures containing oospores the antheridium gives, almost without an exception, the appearance of being pushed up at the base as though the oogonium had penetrated it, but in its younger stage this is not the case, as it is well rounded at the base and shows no sign of pressure. In a majority of cases in this species, where the oogonium appears to emerge from the antheridium, the funnel-shaped base of the oogonium, noted by Pethybridge in his description of *P. erythroseptica*, (6) is often lacking except in very young stages of its formation. In one case where pressure was applied, the antheridium seemed to tear loose from the oospore as if it were wrapped around the stalk (Plate XXIII, figs. 19, 20).

The oogonium and the antheridium apparently come from different filaments. In one or two cases, however, branches that appeared to bear oogonia and antheridia in primordial stages, when traced back for some distance, were found to originate from the same filament but quite a distance apart (Plate XXIII, fig. 5).

Much difference of opinion has existed concerning the relation of the antheridium to the oogonium in this type of oospore. In *P. erythroseptica* Pethybridge (6) advanced the theory that the oogonial incept grew up and pierced the antheridium, growing through it and out on the other side where it appeared as a club-shaped structure and later as a spherical oogonium. This theory was borne out by Murphy (5) in morphological and cytological studies on the same species and this method of formation for *P. erythroseptica* became an established fact. A recent article by Tabor and Bunting (14) on a disease of cocoa and coffee fruits gives added weight to the above conclusions. They find that in mature oospores the separation of oospore from antheridium can be effected by pressure and the depression left indicates that the base of the oospore is within the antheridium.

In the species under observation many instances give the appearance of the antheridium having been pierced by the oogonial stalk. This is especially noticeable in older cultures. The funnel-shaped appearance of the stalk being very apparent in the younger stages (Plate XXIII, fig. 4), but almost entirely disappearing as the oospores mature (Plate XXIII, figs. 6, 8, 13).

In the later stages of the development of the oospore the stalk may be observed from the top to the bottom of the antheridium to be of equal diameter. Sometimes it is even bulged in the center, and often is thinner near the oospore than at the base. Sometimes even what appears to be a curve in the stalk may be seen opposite the middle of the antheridium. In material which contains primordial stages of the oogonium and antheridium, it is not uncommon to see the twisting of one hyphal branch about another, or the swollen knobs with another branch growing closely along its side and curving over the tip, or the mycelium forked and growing around another filament. These conditions have led to the question as to whether the oogonial incept always pierces the antheridium in its formation in this species. Might it not sometimes be formed by some other method, namely, that suggested by Clinton (2) in his work on Phytophthora, that is, by the oogonial branch growing up along the side of the antheridium and forming at the top? Or might it not be formed by twisting about the oogonial stalk and anastomosing as is an occasional occurrence among threads of the mycelium? For this study Van Tieghem cells were used. A thin film of media was spread on flamed cover glasses, and the fungus transferred to this with a sterile platinum needle. Conditions did not seem to be right for full develop-

ment of oospores, however, hence most of the observations were made on ordinary slides from reproducing cultures.

Many and varied experiments were used in an effort to germinate the oospores and in only a few cases did success attend the efforts. The oospore germinates with a thick germ tube with usually a swelling at the base as it emerges from the oospore. In the cases observed the oospore seemed to give rise to mycelium rather than a conidiophore as pictured and described by Rosenbaum (11) for *P. cactorum* (Plate XXIII, fig. 9).

CULTURAL STUDY

In his work on oospore production Clinton (4) tried out many kinds of media in an effort to determine the most satisfactory one for the culture of the species of Phytophthora. After over a thousand cultures of the downy mildews of the potato and lima bean on various nutrients, ranging from potato plugs to synthetic media, he found that the lima bean agar produced the best results.

When the cultural study of the species under consideration was begun the fungus was badly contaminated with bacteria, so the first task was to secure pure cultures. The medium first selected was the ordinary potato agar which is used successfully for the growth of most saprophytic fungi. Ordinary transfers were made with the hope that the fungus would grow more rapidly than the bacteria and that pure cultures might be obtained by transfers from the extreme edges of the growth. This method failed, as the bacteria grew as rapidly as the mycelium and followed out each hyphal thread. Weak lactic acid was then used with success. Pure cultures having been secured, media of several kinds were used for experimentation; potato, oat, lima bean, and tomato agar were used with success.

Oospores were produced in numbers on all media used, but on oat agar very few conidia were ever obtained. Two conditions were noticeable in the production of oospores, namely, the temperature and age of the mycelium. When fresh cultures were kept at a temperature of 8–10° C. they produced oospores in about eight days. They were more sparingly produced as the temperature was increased. Media made with no other ingredient than tomato juice and agar produced luxuriant growth of the fungus even when the acid condition was great enough to soften the agar. Here, too, oospores were often produced in eight days. Under milder conditions of temperature (20° C. or more) six weeks might elapse before their production.

Van Tieghem cells were used freely in the cultural study of conidiophores and the germination of conidia. Small portions of mycelium were transferred to cover glasses and the hanging drop culture method was used. Also flamed cover glasses were spread with a thin film of media and transfers made to these for the study of the oospore. What seemed to be primordial stages in the development of oospores appeared, but for some reason never developed well. This was probably due to the lack of proper amount of oxygen or to the fact that the agar in the cell was not of sufficient depth to give the right conditions for development.

A culture of *P. parasitica* received from Dr. W. S. Beach of Pennsylvania State College, and also one of *P. terrestria* received from Dr. C. D. Sherbakoff of Knoxville, Tennessee, were cultured for comparison. The macroscopic growth of the latter is quite different on all media, growing in tufts over the surface, while the one under consideration makes a smooth growth. The former gives somewhat the tufted appearance also, but is not as noticeably different in this respect. It, however, produces largely chlamydospores with but few conidia and no oospores were observed on any of the media used.

Much work was done in an effort to germinate oospores. Van Tieghem cells with hanging drops were first made of oospores of different ages, ranging from three months to a year old. These were subjected to varying degrees of temperature without obtaining germination. Tubes were then treated with very dilute acids and transfers made to Van Tieghem cells with like results. Peroxide solution was used in tubes, then removed and transfers made. The last two tests were made with the hope that if the oospore wall were softened or if given greater amount of oxygen, the spores would germinate. Then tubes with oospores were placed in the outdoor conditions, some with sterile water added and some without, and left for one, two, three, four and eight weeks. In tubes left out under these conditions for four or eight weeks and then sterile water placed in them, when warm spring weather came, some few oospores germinated, but none of the work on the oospore germination could be considered very successful.

PROOF OF PATHOGENICITY

That the organism is pathogenic in character is shown by its prompt action on plants inoculated with the fungus having abundant conidial growth. Large numbers of young tomato plants were inoculated by

spraying with sterile water containing conidia, and when these were left under a bell jar twenty-four hours and kept reasonably moist thereafter they showed a characteristic black wilt of all parts above the inoculation (Fig. 1). In some cases small pieces of agar containing the fungus were placed in the axil of leaves. Each leaf, with all parts above, gave evidence of the effect in forty-eight hours. When the fungus was placed on the soil at the base of young plants, the plants dampened off at the surface of the soil. In cases where older stems were inoculated, a much longer time (three or four days) elapsed before signs of infection appeared.

FIG. 1. The effect on the tomato plant forty-eight hours after being inoculated with *Phytophthora mexicana*. Right, the control.

On the tomato fruit in two days a water-soaked region appeared around the point of inoculation and in a week's time a rot had destroyed the entire fruit. The rotting in these instances did not show rings as in the disease of fruit caused by *P. terrestria*, but produced rather an even shriveling of the entire fruit by a white mycelial growth over the entire

surface. Cultures made from the center of the fruit produced luxuriant growth of the mycelium together with many conidia and oospores.

IDENTIFICATION

In comparing the fungus under consideration with known species of Phytophthora only those species need to be considered which have a similar position of the antheridia at the base of the oogonia. The following species show this relationship: *P. phaseoli* Thax., *P. parasitica* Dastur, *P. colocasiae* Racib., *P. infestans* (Mont.) de Bary, *P. erythroseptica* Pethyb., *P. arecae* Colem., *P. cryptogea* Pethyb., *P. terrestria* Sherb., *P. allii* K. Swadii, *P. melongenae* K. Swadii, and *P. capsici* Leon. In making this comparison *P. cryptogea* and *P. erythroseptica* can be eliminated by the lack of conspicuous conidial papillae; *P. colocasiae* by its short, blunt papillae; *P. infestans* by the scanty production of oospores; *P. arecae* by the presence of a vesicle into which the zoospores are deposited on the germination of the conidia; *P. parasitica*, *P. terrestria*, *P. allii* and *P. melongonae* by the smaller size of the oospores; *P. capsici* by the tuberous appearance produced on hard potato agar as well as the smaller sized oospores and the larger conidia; *P. phaseoli* by the smaller oospores, more variable conidia and conidiophores, as well as the fact that the oospores are not produced readily on an acid medium, while the one under consideration grows luxuriantly on media that is decidedly acid. Since the organism studied does not seem to be identical with any of the above forms, it has been described as follows as a new species.

Phytophthora mexicana HOTSON & HARTGE

Mycelium irregularly branched, hyaline, non-septate when young, often septate and empty when old, sometimes short and knobby; conidiophores at first simple and bearing a single apical conidium but eventually becoming long and much branched; conidia terminal but not inhibiting the growth of the conidiophore, varying greatly in size, more or less inversely pear-shaped, 16–33 μ by 16–77 μ, sometimes measuring twice as long as wide; papillae prominent, usually forming zoosporangia; zoospores ovoid, depressed slightly on one side, germinating immediately by germ tube; chlamydospores intercalary, sparingly produced, almost spherical 28–44 μ in diameter, yellowish when old, germinating by germ tube; oospores yellowish at maturity, nearly spherical, 24–37.4 μ in diameter; episporium 4.4 μ thick; antheridium terminal or lateral, somewhat spherical or ovoid, surrounding the base of the oogonium as if penetrated by it as in *P. phaseoli* Thaxter.

Hab. On the fruit *Lycopersicum esculentum* Mill from Mexico.

Mycelio ramoso irregulariter, hyalino, non-septato recentiore sed vetustiore multi-septato vacuatoque, interdum brevi et nodoso; conidiophoris primo unicis et sustinentibus conidium unum in apice sed tandem longo ramosoque; conidiis terminalibus sine inhibitionis auctus conidiophori, variabili magnitudine, paene in forma inversi pyri, 16–33 μ × 16–77 μ, interdum longioribus duplo quam latioribus, papillis prominentibus, plerumque formatibus zoosporangia; zoosporis ovoideis, depressis paulum in uno latere, germinantibus statim hyphis; chlamydosporis intercalaribus sed paucis, paene sphaeroideis 28–44 μ diam., luteis vetustioribus germinantibus statim hyphis; oosporis luteis vetustioribus, sphaeroideis 24–37 μ diam.; episporis 4.4 μ densis; antheridiis terminalibus et lateralibus sphaeroideis aut ovoideis, circumambientibus bases oogoniorum quasi penetratis ab eis sic ut in *P. phaseoli* Thaxter.

Hab. In fructibus *Lycopersici exculenti* Mill, Mexico, Amer. bor.

SUMMARY

1. A fungus isolated from tomatoes shipped from Mexico proved on cultural study to be a Phytophthora. The mycelium averages 4.4 μ in diam., the conidia measure 16–33 × 16–77 μ. The oospores are large, the greater number of them measuring 37 μ. Occasionally chlamydospores are produced.

2. The sexual and asexual spores have been found on all media used.

3. Inoculations of young and old tomato plants produced a black wilt. Inoculation of the fruit caused a destructive rot in a week's time.

4. The organism studied does not seem to be identical with any of the known species of Phytophthora, and the name *Phytophthora mexicana* has been proposed.

The authors of this paper are indebted to Dr. W. S. Beach, Dr. S. D. Sherbakoff and Dr. M. W. Gardiner for cultures of Phytophthora.

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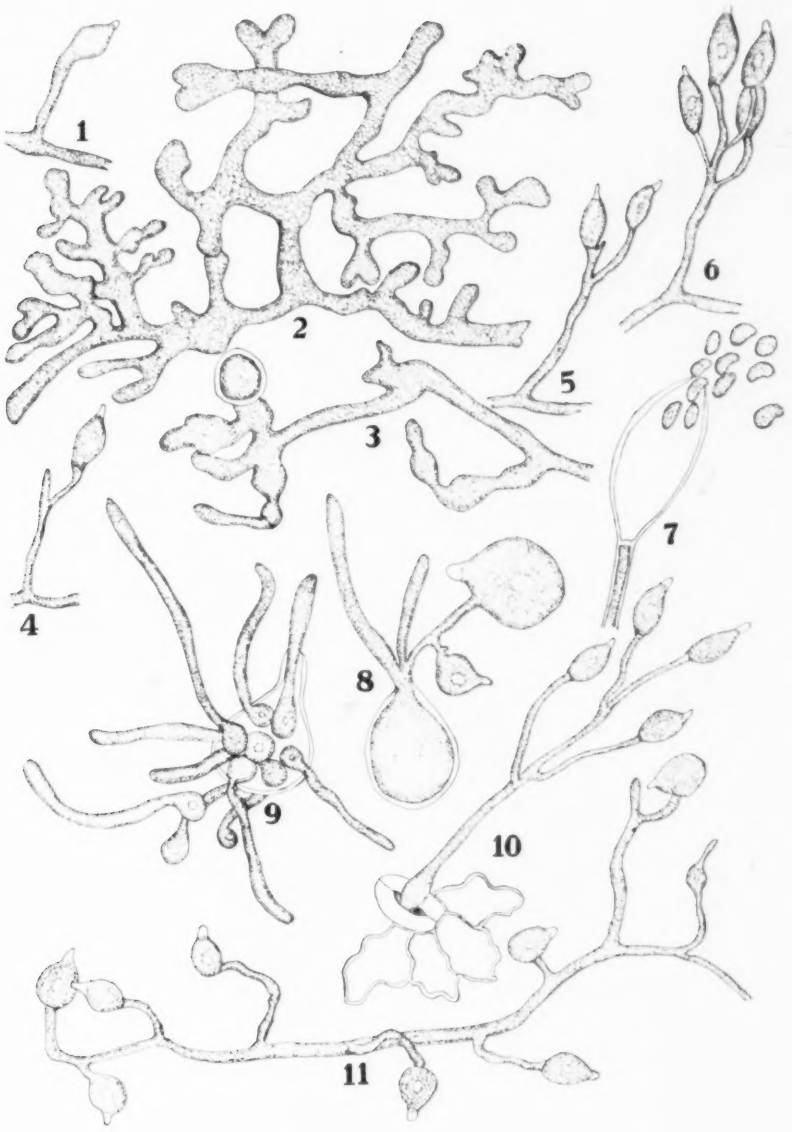
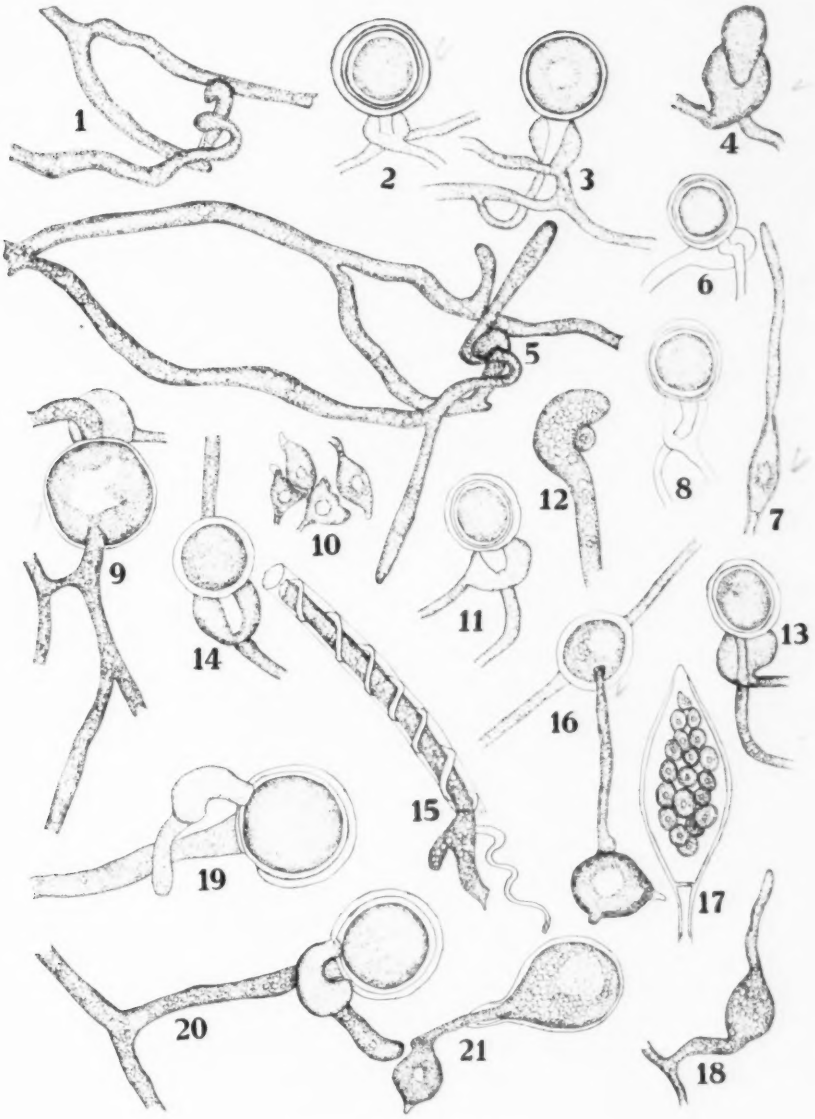


PHYTOPHTHORA MEXICANA

Fig. 1.—A young conidium. Fig. 2.—The knobby character of the mycelium. Fig. 3.—The knobby mycelium producing oospores. Fig. 4.—A conidiophore showing the first conidium. Fig. 5.—A young conidiophore showing two conidia. Fig. 6.—A young conidiophore showing several conidia. Fig. 7.—A conidium (zoosporangium) discharging zoospores. Fig. 8.—A conidium germinating by sending out germ tubes. Fig. 9.—A conidium showing zoospores germinating by germ tubes in the interior. Fig. 10.—A conidiophore coming out through a stoma of a leaf. Fig. 11.—A conidiophore showing several conidia.



PHYTOPHTHORA MEXICANA

Fig. 1.—Probably a primordial stage in the formation of an oogonium and antheridium. Figs. 2, 4.—Stages in the development of the oospore. Fig. 5.—A probable primordial stage in the formation of an oogonium and antheridium showing that the two may come from branches of the same hypha. Figs. 6, 8, 11, 13, 14.—Oospores. Fig. 7.—A conidium germinating by a single germ tube. Fig. 9.—A germinating oospore. Fig. 10.—Types of conidia. Fig. 12.—Probably a primordial stage of an oogonium and antheridium. Fig. 15.—A hypha growing in a spiral vessel. Fig. 16.—A chlamydospore germinating by a single germ tube and immediately forming a conidium. Fig. 17.—Formation of zoospores in a germinating conidium. Fig. 18.—A young chlamydospore. Fig. 20.—An oospore and antheridium after exerting a slight pressure. Fig. 19.—The same as Fig. 20 after more pressure had been exerted. Fig. 21.—A conidium germinating by a single germ tube and immediately forming another conidium.

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TOXICITY STUDIES WITH SOME COPPER FUNGICIDES

W. L. Doran

The object of the experiments here described is to compare the susceptibility of several fungi to copper.

In the experiments described in the present paper the author has used the following fungicides: copper sulphate, neutral copper acetate, Burgundy mixture, Bordeaux mixture of various types, cuprammonium sulphate and Johnson's mixture. Spores of the following fungi were used as test objects: *Gymnosporangium clavipes, Gymnoconia interstitialis, Uromyces caryophyllinus, Cronartium ribicola, Puccinia antirrhini, Alternaria solani,* and *Venturia inaequalis.*

In preparing the fungicides the following methods were used: The Bordeaux mixtures, in which a weighed amount of lime was used, were made by adding the stock solution of lime to the diluted copper sulphate. Neutral Bordeaux mixture was prepared by adding to a weak solution of copper sulphate, milk of lime until a mixture alkaline to phenolphthalein was obtained. Zucker's Bordeaux mixture was made by adding saccharose to a wash containing copper sulphate and quick lime in the ratio 1:0.5 in amount equivalent to the copper sulphate taken. Perret's Bordeaux mixture was made by adding to a wash containing copper sulphate and quick lime in ratio 1:0.5 an amount of molasses equivalent to the copper sulphate used. Burgundy mixture was prepared by combining copper sulphate and sodium carbonate crystals in the ratio 1:1.84. The wash was made by adding the stock solution of sodium carbonate to a weak solution of copper sulphate. Cuprammonium sulphate (*eau celeste*) was prepared by adding ammonium hydroxide to a solution of copper sulphate in sufficient quantity to give a clear solution. Johnson's mixture was prepared according to the original formula, that is, copper sulphate and undecomposed ammonium carbonate were used in the ratio of 1:2.

The toxicity tests were made following the method of Reddick and Wallace (7), which has been described in detail by Wallace, Blodgett and Hesler (9). It consists essentially in the germination of fungous spores in drops of water on a glass surface which has been previously sprayed with the fungicide to be tested and dried. The spores were placed under optimum conditions for their germination. Clark (4) concluded that a fungus presents its greatest resistance to the action of fungicides when the temperature is at the optimum for spore germination. We cannot consider a fungicide toxic unless it is toxic under optimum

conditions for spore germination. The writer found that when the temperature was near the minimum or maximum for germination of the spores some dilutions of fungicides were toxic that were not toxic at optimum temperatures.

The results given in this paper are expressive of the point of inhibition of germination rather than of the death point of the spores. To determine the death point, requires the transfer of the spores to a fresh culture free from the fungicide. This was considered unnecessary; for Clark (3) found that, if the germination of spores is inhibited by a fungicide, they will in time be killed by it.

The data given are in each case the mean of at least five experiments, exclusive of those in which the germination of the spores in the check showed poor viability which were disregarded as untrustworthy.

In order that the copper fungicides may exert their toxicity, sufficient water must be present to act as a solvent. When the spores of *Alternaria solani* were placed in water on a slide sprayed the day before with a 1.0 per cent solution of copper acetate, the germination of the spores was prevented. But when the spores of *Alternaria solani* were placed dry on a dry slide sprayed the day before with the same concentration of copper acetate, spore germination was not prevented. They germinated as well as on a dry slide in the absence of any fungicide. In the same way, it was found that a copper sulphate solution (0.125 per cent copper) which prevents the germination of the aeciospores of *Gymnosporangium clavipes* when the spores are placed in water upon the sprayed slide, does not prevent the germination of these spores when they are placed dry upon a dry slide which has previously been sprayed with the same concentration of copper sulphate solution. Although the lethal concentration of copper sulphate solution for *Gymnosporangium clavipes* is, in the presence of water, not more than 0.125 per cent copper, it was found that a solution containing 0.5 per cent copper did not prevent germination of these spores when they were placed dry on the dried sprayed slide, so that the only available moisture was water vapor. Germination at this double concentration was, however, retarded.

EXPERIMENTS WITH COPPER SULPHATE

In table 1 are shown the results with copper sulphate. *Rhizopus nigricans* is eight times as susceptible to it as the most susceptible representative of the Uredinales and sixty-four times as susceptible as the spores of the most resistant rust fungus tested. The indications are that the Uredinales as a group are very resistant to the action of copper sulphate.

The urediniospores of *Cronartium ribicola* are somewhat more resistant to the toxic action of copper sulphate than are the aeciospores of this fungus. It appears that, while a solution containing 0.5 per cent copper sulphate might protect Ribes against first infection from the pine, it would require a solution containing not less than 1.0 per cent copper sulphate to protect Ribes from infection from Ribes, that is, by urediniospores. It is doubtful if these concentrations of copper sulphate could safely be used on Ribes foliage. Butler (1) found copper sulphate solution containing only 0.25 per cent copper sulphate toxic to the foliage of coleus, tomato, cauliflower, bean, oxalis and pelargonium.

A study of table 1 shows that the susceptibility of any one fungus to copper sulphate solutions of various concentrations is by no means uniform. The percentages of germination of all spores tested drop rapidly as the copper content increases up to 0.0039–0.0317 per cent. Beyond these concentrations of copper only the urediniospores and aeciospores of *Cronartium ribicola* and the urediniospores of *Uromyces caryophyllinus* continue to germinate in considerable numbers.

EXPERIMENTS WITH NEUTRAL COPPER ACETATE

As shown in table 2, the fungous spores tested rank as follows in their resistance to neutral copper acetate. The most resistant are the urediniospores of *Puccinia antirrhini*, and the urediniospores of *Uromyces caryophyllinus*; next comes *Alternaria solani;* while *Rhizopus nigricans* and *Botrytis cinerea* are the least resistant. Again, the Uredinales appear to be markedly resistant to copper.

TABLE 1—*Toxicity of copper sulphate.*

Per cent copper in solution	Relative number of spores germinating					
	R. nigricans	*G. clavipes*	*G. interstitialis* Aeciospores	*U. caryophyllinus* Aeciospores	*C. ribicola* Urediniospores	*C. ribicola* Urediniospores
0.0000	100	100	100	100	100	100
0.0039	6	...	...	...	...	...
0.0079	0	15	...	69	25	...
0.0158	0	...	...	29	20	...
0.0317	0	0	6	5	20	15
0.0635	0	0	0	2	7	9
0.127	0	0	0	1	0	3
0.254	0	0	0	0	0	0

TABLE 2—*Toxicity of neutral copper acetate.*

Per cent copper in solution	Relative number of spores germinating				
	P. antirrhini	*U. caryophyllinus*	*A. solani*	*R. nigricans*	*B. cinerea*
0.0000	100	100	100	100	100
0.0049					0
0.0099			16.5	0	0
0.0199			11	0	0
0.0398	3	3	3	0	0
0.0796	2	3	0	0	0
0.1592	0	0	0		0
0.3185	0	0	0		0
0.6370		0			

EXPERIMENTS WITH BURGUNDY MIXTURE

The fungous spores used in the experiments with Burgundy mixture show the following degree of resistance, the most resistant named first: urediniospores of *Cronartium ribicola*, urediniospores of *Puccinia antirrhini; Alternaria solani*, aeciospores of *Cronartium ribicola*, aeciospores of *Gymnoconia interstitialis*, and conidia of *Venturia inaequalis*. The results are given in table 3.

Attention is here called to the extreme susceptibility to copper in Burgundy mixture of *V. inaequalis*. That this is the normal behavior of this fungus against copper will appear subsequently. A Burgundy mixture containing only one-eighth pound of copper sulphate in fifty gallons entirely prevents germination of the conidia of this fungus.

Here, as in the case with copper sulphate, the urediniospores of *C. ribicola* are more resistant to the action of the fungicide than are the aeciospores of this fungus. A concentration of Burgundy mixture sufficient to protect the host against uredinial infection by this fungus would probably be dangerous to the host plant.

Of the representatives of the Uredinales studied, the aeciospores of *G. interstitialis* are the most susceptible to the copper in Burgundy mixture. This would perhaps make possible the control of orange rust of raspberries by spraying with Burgundy mixture if it were not for the perennial habit of the mycelium in the cane.

Burgundy mixture shows up decidedly better against *P. antirrhini* than does Bordeaux mixture. Doran (5) has already shown that Bordeaux mixture does not prevent the germination of the spores of this fungus even when 1.0 per cent copper is present.

TABLE 3—*Toxicity of Burgundy mixture 1:1.84.*

Per cent copper in solution	Relative number of spores germinating					
	V. inaequalis	*A. solani*	*G. interstitialis*	*C. ribicola* Aeciospores	*C. ribicola* Urediniospores	*P. antirrhini*
0.0000	100	100	100	100	100	100
0.0079	0	...	...	...	...	...
0.0158	0	...	...	...	...	...
0.0317	0	62	5	...	...	...
0.0635	0	35	0	9	79	57
0.1270	0	10	0	0	35	21
0.2540	0	0	0	0	11	6
0.5080	.				0	0

TABLE 4—*Toxicity of Bordeaux mixture 1: 1.*

Per cent copper in solution	Relative number of spores germinating			
	A. solani	*V. inaequalis*	*G. clavipes*	*C. ribicola*
0.0000	100	100	100	100
0.0038	..	6	...	...
0.0079	..	2	...	...
0.0158	..	0	...	...
0.0317	81	0	...	...
0.0635	63	0	20	29
0.127	14	0	19	21
0.254	1	0	17	10
0.508	0	0	7	10

TABLE 5—*Toxicity of Zucker's Bordeaux mixture to the conidia of V. inaequalis.*

Per cent copper in solution	Relative number of spores germinating
0.0000	100
0.0019	4
0.0039	0
0.0079	0
0.0158	0
0.0317	0
0.0635	0
0.127	0
0.254	0

EXPERIMENTS WITH BORDEAUX MIXTURE 1:1

The results with Bordeaux mixture are summarized in table 4. Bordeaux mixture is toxic to the spores of *Alternaria solani* when 0.508 per cent copper is present. It is toxic to the conidia of *Venturia inaequalis* when as little as 0.0079 per cent copper is present. It is not toxic to the aeciospores of *Gymnosporangium clavipes* nor to the urediniospores of *Cronartium ribicola* when as much as 0.508 per cent copper is present.

Of these four fungi, *Venturia inaequalis* is the most susceptible and *Cronartium ribicola* is the most resistant to the fungicidal action of Bordeaux mixture. It is evident that the dissemination of *Cronartium ribicola* from currant to currant cannot be prevented by spraying with Bordeaux mixture even when this is prepared to contain twice the usual amount of copper. Unit for unit of copper, Burgundy mixture is more toxic than is Bordeaux mixture to the urediniospores of *C. ribicola.*

EXPERIMENTS WITH BORDEAUX MIXTURES 1:5 AND 1:3.3

Tests were made of the toxicity to the conidia of *Venturia inaequalis* of the Bordeaux mixture described by Sanders and Brittain (8). Two Bordeaux mixtures were used, one made up at the rate of two pounds, and the other at the rate of three pounds of copper sulphate and ten pounds of lime in forty gallons of water. Bordeaux mixtures 1:5 and 1:3.3 have a fungicidal efficiency against this fungus which is at least equal to that of ordinary Bordeaux mixture.

EXPERIMENTS WITH ZUCKER'S BORDEAUX MIXTURE

In table 5 are given the results of the tests of the toxicity of Zucker's sugar-Bordeaux mixture to the conidia of *Venturia inaequalis.* The mixture is toxic to these spores when as little as 0.0039 per cent copper is present. Therefore, the toxicity of Bordeaux mixture 1:0.5 to the conidia of *V. inaequalis* is increased four times by the addition of sugar.

TABLE 6—*Toxicity of Perret's Bordeaux mixture to the conidia of V. inaequalis.*

Per cent copper in solution	Relative number of spores germinating
0.0000	100
0.0019	5
0.0039	1
0.0079	0
0.0158	0
0.0317	0
0.0635	0
0.127	0
0.254	0

TABLE 7—*Toxicity of cuprammonium sulphate.*

Per cent copper in solution	Relative number of spores germinating		
	C. ribicola	*U. caryophyllinus*	*B. cinerea*
0.0000	100	100	100
0.0079	...	...	...
0.0158	...	75	0
0.0317	...	56	0
0.0635	6	36	0
0.1270	0	32	0
0.2540	0	10	0

EXPERIMENTS WITH PERRET'S BORDEAUX MIXTURE

The results of the tests of the toxicity of Perret's Bordeaux mixture to the conidia of *Venturia inaequalis* are shown in table 6. It is twice as toxic to these spores as is Bordeaux mixture 1:1, being intermediate in this respect between Bordeaux mixture 1:1 and Zucker's Bordeaux mixture.

EXPERIMENTS WITH CUPRAMMONIUM SULPHATE

The results of the toxicity tests with cuprammonium sulphate are shown in table 7. *Botrytis cinerea* is most susceptible to this fungicide, the germination of its spores being prevented when as little as 0.0079 per cent copper is present. The germination of the aeciospores of *Cronartium ribicola* is prevented by cuprammonium sulphate containing 0.127 per cent copper. This strength would possibly be toxic to the foliage of the currant, for Butler (1) found cuprammonium sulphate of this concentration to be toxic to coleus and bean. The germination of the urediniospores of *Uromyces caryophyllinus* is not prevented even when 0.254 per cent copper is present, and the use of cuprammonium sulphate for the control of carnation rust is evidently out of the question.

When cuprammonium sulphate dries on a sprayed surface there is deposited, according to Butler (1) a basic sulphate of copper and mixed with it there is a little ammonium sulphate in the ratio of 1:0.35.

What part, if any, of the toxicity of cuprammonium sulphate is due to the above mentioned ammonium sulphate was next determined. Solutions of ammonium sulphate containing from 0.0312 to 2.0 per cent of the salt were found to be non-toxic to the spores of *Botrytis cinerea*. Solutions of these concentrations did not prevent germination of the

urediniospores of *Uromyces caryophyllinus*, although the most concentrated reduced their germination 40 per cent. Evidently ammonium sulphate is not itself alone, toxic at the strength at which it is found in cuprammonium sulphate.

TABLE 8—*Toxicity of Johnson's mixture.*

Per cent copper in solution	Relative number of spores germinating			
	P. antirrhini	*U. caryophyllinus*	*C. ribicola* urediniospores	*C. ribicola* aeciospores
0.0000	100	100	100	100
0.0317	...	...	...	6
0.0635	45	9	4	0
0.1270	9	3	0	0
0.2540	0	0	0	0

EXPERIMENTS WITH JOHNSON'S MIXTURE

As is shown in table 8, the fungous spores tested rank as follows as regards their resistance to Johnson's mixture (6), the most resistant being named first; urediniospores of *Puccinia antirrhini*, urediniospores of *Uromyces caryophyllinus*, urediniospores of *Cronartium ribicola*, and aeciospores of *C. ribicola*. The good showing made by Johnson's mixture may be due in part to the ammonium sulphate present. Butler (1) found that the presence of ammonium sulphate increases the toxicity of a copper wash to *Plasmopara viticola*.

RELATIVE SUSCEPTIBILITY OF FUNGI TO COPPER

We are now in a position to determine how some of the pathogenic fungi compare in susceptibility to copper fungicides. Clark (3) did pioneer work along this line; and of the fungi with which he worked those found to be most resistant to copper sulphate were the molds Aspergillus, Sterigmatocystis, Botrytis, and Penicillium. Toward the fungicides with which Clark (2) worked, the fungi studied proved increasingly resistant in the following order: Botrytis, Oedocephalum, Penicillium and Aspergillus.

The results obtained by the writer are sufficient to make possible a comparison of the susceptibility to copper fungicides of aeciospores and urediniospores of Uredinales. These are summarized in table 9, a study of which shows that the average lethal concentration in per cent copper

for aeciospores is 0.0834 and for urediniospores it is 0.2746. In other words, it requires 3.2 times as much copper to prevent the germination of urediniospores as it does to prevent the germination of aeciospores. It is probable that no disease of plants dependent upon urediniospores for its dissemination can be successfully combatted by the use of copper fungicides, since sufficient copper to check the fungus would also injure the host plant.

The average lethal concentration for all spores of Uredinales was 0.179 per cent copper as compared with a lethal concentration of 0.088 per cent copper for all other spores. The spores of Uredinales are twice as resistant to copper as the spores of other fungi.

The order of resistance to copper fungicides is as follows with copper sulphate: urediniospores of *C. ribicola*, aeciospores of *C. ribicola*, urediniospores of *U. caryophyllinus*, aeciospores of *C. ribicola*, aeciospores of *G. interstitialis*, aeciospores of *G. clavipes*, spores of *R. nigricans*; with Bordeaux mixture: urediniospores of *C. ribicola*, aeciospores of *G. clavipes*, spores of *A. solani*, conidia of *V. inaequalis*; with copper acetate: urediniospores of *P. antirrhini*, (equal to) urediniospores of *U. caryophyllinus*, spores of *A. solani*, *R. nigricans*, *B. cinerea*; with cuprammonium sulphate: urediniospores of *U. caryophyllinus*, aeciospores of *C. ribicola*, urediniospores of *U. caryophyllinus*.

TABLE 9—*Susceptibility of aeciospores and urediniospores to copper.*

Fungus	Fungicide	Lethal concentration	
		Aeciospores	Urediniospores
		Per cent copper	Per cent copper
G. clavipes	Copper sulphate	0.0317	.....
G. interstitialis	Copper sulphate	0.0635	.....
U. caryophyllinus	Copper sulphate	.....	0.2540
C. ribicola	Copper sulphate	0.1270	0.2540
U. caryophyllinus	Copper carbonate	.....	1.1480
P. antirrhini	Copper acetate	.....	0.1592
U. caryophyllinus	Copper acetate	.....	0.1592
G. interstitialis	Burgundy mixture	0.0570	.....
C. ribicola	Burgundy mixture	0.1140	0.4580
P. antirrhini	Burgundy mixture	.....	0.4580
P. antirrhini	Johnson's mixture	.....	0.2540
C. ribicola	Johnson's mixture	0.0635	0.1270
U. caryophyllinus	Johnson's mixture	.....	0.2540
C. ribicola	Cuprammonium sulphate	0.1270	.....
U. caryophyllinus	Cuprammonium sulphate	.....	0.5000

spores of *P. antirrhini*, spores of *B. cinerea*; with Johnson's mixture: urediniospores of *U. caryophyllinus*, (equal to) urediniospores of *P. antirrhini*, urediniospores of *C. ribicola*, aeciospores of *C. ribicola*.

The average amount of copper necessary to prevent germination of the conidia of *V. inaequalis* is 0.0145 per cent as compared with an average of 0.133 per cent for all other spores. The apple scab fungus is about nine times as sensitive to copper as the average of the other spores tested.

SUMMARY

The Uredinales are twice as resistant to the toxic action of copper as are other fungi. Urediniospores of these fungi are three times as resistant to copper as are aeciospores. Spraying with copper fungicides for the prevention of infection by urediniospores is probably impracticable. The indications are that Johnson's mixture will succeed against urediniospores if any copper fungicide will.

The conidia of *V. inaequalis* are extremely susceptible to copper in any form, being nine times as sensitive to it as the average of all other spores studied.

Bordeaux mixtures of the 2–10–40 and 3–10–40 formulae were not found to differ from Bordeaux mixture of the 4–4–50 formula in toxicity to the conidia of *Venturia inaequalis*.

By the addition of sugar, at the rate of 4 pounds in 50 gallons of 4–2–50 Bordeaux mixture, the toxicity of the wash to the conidia of *Venturia inaequalis* is increased four times.

Bordeaux mixture of Perret's formula, prepared by the addition of molasses, is twice as toxic as is ordinary Bordeaux mixture to the conidia of *Venturia inaequalis*.

Aeciospores of *G. clavipes* are so resistant to the copper in Bordeaux mixture that spraying the conifer host of this, and presumably other Gymnosporangiums, with this fungicide would prove of doubtful value.

Burgundy mixture surpasses Bordeaux mixture in its toxicity to the spores of *A. solani*.

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THE PRODUCTION BY FUSARIUM SOLANI OF A TOXIC EXCRETORY SUBSTANCE CAPABLE OF CAUSING WILTING IN PLANTS

T. FAHMY[1]

Many plants, such as cotton, potatoes, banana trees, carnations and others are liable to be attacked by Fusarium. The parasite seems to gain entrance through the root system, developing mainly in the vascular tissue.

According to some authors[2] the fungal hyphae are supposed to cause a mechanical plugging of the vessels, and bring about the wilting of the host plant by cutting off its water supply; or as MacMillan[3] puts it: "That the fungus has created a physiological drouth which has extended over a long period."

Butler[4] states that in the case of pigeon pea wilt caused by *Fusarium udum*: "Though the water supply to the roots may be ample, its passage to the green parts is impeded by the choking of the vessels; this may be sufficient to produce the same effect as severe drought, but it is possible that poisonous substances excreted by the fungus may also play an important part."

It has also been stated, in the case of *F. cubensie*, causing banana wilt,[5] that the fungus tissue in the vessels is not present in quantity sufficient to cause serious obstruction to the passage of water.

It appears that the blocking by the fungus of the vascular tissue of the host to any serious extent is doubtful; and the presence of some factor other than the "mechanical plugging" is highly probable. This factor, responsible for the wilting, may be in the nature of diffusible substances injuring the living plant, and causing it to wilt. Such substances may be enzymes or other excretory products produced by the fungus during its growth in the plant.

[1] The work here published was done in 1920 at the Imperial College of Science, London.

[2] Massee, G. Diseases of cultivated plants and trees *pp. 490–496*. Edition 1915.

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The object of the present investigation was to find out whether or not;

(1)—Fusarium, such as *Fusarium solani*, produces toxic substances capable of causing wilting in plants.

(2)—These substances, if present, are of enzymic nature.

THE FUNGUS

A slant of potato agar[1] was inoculated with *F. solani* from a stock culture.[2] This slant was then incubated at 25° C. for 24 days after which it was examined and had the following characters.

Fusiform macroconidia and globose chlamydospores present, of the following measurements:—

0—Septate (8–10) × (4–5) μ
1—Septate (18–24) × (5–6) μ
2—Septate (26–28) × (5–6.5) μ
3—Septate (28–36) × (6.5–7.5) μ
4—Septate (40–47) × (6.7–8) μ
very few
Chlamydospores 6 × 8 μ

The aerial mycelium, well developed, olive white in color, substratum buff rather than olive.

A dilute spore suspension was made in sterile water, and a drop of this was placed in the centre of a sterile petri-dish, by lifting its cover just enough to permit the drop to be deposited, the petri-dish was then inverted and the drop examined under the microscope with the ⅔ objective. Nine spores were found present in this drop; melted agar at 40° C. was poured into the petri dish, and incubated at 25° C. for three days, after which it was examined. Nine centres of growth were apparent to the naked eye; one of these was transplanted on to a sterile petri-dish containing sterile potato agar, and incubated at 25° C. for 24 days. When examined it was found to be similar in all its characters to the former culture.

In this way a pure culture of *F. solani* raised from a single spore was obtained, one strain only being present.

[1] The potato agar was prepared in the following way: —a) —400 grms. of peeled potatoes were steamed for an hour with a little water, then passed through cheese cloth. b)—15 grms. of agar in 500 ccs. of water were cooked at 110° for 30 minutes; to this the potato was added and the whole made up to 1 litre, well mixed, tubed and sterilized by intermittent heat, to prevent the potato from darkening.

[2] The original culture was obtained from Dr. Horne

THE EXPERIMENT

The media. The first thing required was a liquid medium on which the fungus could grow freely, and when in a pure sterile state would not bring about wilting of stems, such as those of broad bean plants, placed in it.

Turnip juice medium. Turnip juice was first tried as medium: 400 grams of white peeled turnips were cut up into small pieces, placed in a flask, plugged, and, without the addition of any water steamed for two hours and a half; the juice was then extracted without the addition of any water, and 240 ccs. of pure turnip juice were obtained. Solutions were made up containing the following percentage of the pure turnip juice:—24 per cent, 20 per cent, 15 per cent, 12½ per cent, and 5 per cent. All were autoclaved for 20 minutes at 120° C.

The fungus grew well on all these solutions, but when stems of broad bean four weeks old were placed in the different solutions of the turnip juice, wilting occurred within a few hours in the two stronger solutions, namely the 24 per cent and the 20 per cent. The wilting of the bean stems occurred earlier and was more pronounced than that of similar bean stems placed in the filtrate of a culture of the fungus after two weeks growth. For this reason the turnip juice as a medium was abandoned.

Richard's solution. The next medium to be tried was Richards Solution, the composition of which is:—

Potassium Nitrate (KNO_3)	10 grms.
Potassium Hydrogen phosphate (KH_2PO_4)	5 grms.
Magnesium sulphate ($MgSO_4$)	2.5 grms.
Iron Chloride ($FeCl_3$)	20 mgrms
Cane Sugar ($C_{12}H_{22}O_{11}$)	50 grms.
Distilled water	1,000 cc.

These quantities were dissolved and autoclaved.

The fungus grew very well on this medium, in fact much better than it did on turnip juice medium, but the pure sterile Richards' solution produced wilting of broad bean within 5–6 hours. To avoid this effect the medium was diluted and it was found that by using 1,500 cc. of water instead of the original 1,000 cc., as given in Eyres' bacteriological technique, wilting was entirely avoided; in fact broad beans placed in such a medium remained fresh for 15 days and even showed marked growth. The fungus grew equally well on this dilute medium, which, being the most satisfactory, was chosen to grow the fungus for this investigation.

Growing the fungus. A number of 500 cc. conical flasks, each containing 200 cc. of Richards' solution were autoclaved for 20 minutes at 120° C. When cold they were inoculated with the mycelium of the fungus grown on potato agar,[1] and incubated at 25° C. A week later an equal number of similar flasks was inoculated and incubated at the same temperature, and still a week later the operation was repeated. After a period of three weeks from the first lot of inoculations, the flasks were taken out of the incubator and each lot separately filtered under sterile conditions. In this way the filtrates obtained were of cultures one week, two weeks and three weeks old.

In each case the filtrate was divided into two portions; one was boiled for a few seconds, and when cold, sterile air was passed through it to replace that which was lost during the boiling. The other part was left unboiled. The object of the boiling was to destroy any thermolabile substances such as enzymes which may have been present, leaving only those which are thermostable.

Solutions of half concentration were made from the boiled and unboiled filtrate by diluting them with distilled water.

A number of test tubes were filled equally with the different solutions, others with distilled water, and others with sterile Richards' solution—the two latter types to serve as controls. In each of these tubes a stem of broad beans was inserted. Besides the water and Richards' solution used as control, bean stems were inserted in empty test tubes. In all cases the bean stems were plugged round with cotton wool.

The broad beans used were grown for three weeks in the greenhouse after being germinated, when used they were 5 inches long and had three pairs of well developed leaves.

The different test tubes with the bean stems were placed in the greenhouse at about 11 a. m. in the morning. The temperature during the months of April and May—during which time the experiment was repeated six times with the same result—was between 60° and 70° F.

The time required for the first signs of wilting to be apparent differed slightly with the temperature of the greenhouse.

The following are the maximum and minimum time required for broad bean stems to wilt in the different filtrates:—

Filtrate	1 week old	2 weeks old	3 weeks old
Boiled Filtrate	20–24 hours	4– 6 hours	2–4 hours
Unboiled Filtrate	20–30 "	4– 8 "	2–4 "
Boiled Fte. ½ strength	40–48 "	6–10 "	4–6 "
Unboiled Fte. ½ strength	40–50 "	6–10 "	4–6 "

[1] The fungus was grown from the single spore culture obtained on potato agar plates.

Controls	
Pure Richards' solution	12–15 days
Distilled water solution	3– 7 days
Air	1– 3 hours

Though the effect of the boiled and unboiled filtrate appeared at about the same time, the bean stems in the unboiled showed the first signs of blackening at the cut end, while often the very earliest signs of wilting appeared in the boiled filtrate. Wilting due to the filtrate, whether boiled or unboiled, appeared first in the two lower leaves, the upper ones remaining apparently fresh, though for a short time only; they soon wilted in a similar manner. The bean stems in the empty test tubes collapsed, wilting uniformly in all the leaves. If, however, these were placed in water even after very marked wilting, they recovered, while those in filtrates never recovered after such treatment.

The filtrate. The filtrate was a clear liquid varying from light to darker shades of amber according to the age of the culture. It had no disagreeable smell and was distinctly alkaline in reaction. When boiled with soda lime, free ammonia was given off.

The following are the average results obtained when the filtrate was titrated with HCl $\frac{N}{10}$, using sodium alizarine sulphate as indicator:—

Filtrate of culture 1 week old: 20 cc. = 3 cc. of $\frac{N}{10}$ HCl or an alkalinity of − 15 (Fuller's)[1]

The − 15 for example means that exactly 15 cubic centimeters of normal acid N/1 must be added to a litre of the substance to render it neutral to the indicator used, and correspondingly + 24 means that the substance is acid and that 24 cubic centimeters of normal alkali N/1 would be needed to be added to the litre to bring it to the neutral point.

Filtrate of culture 2 weeks old: 20 cc. = 12 cc. $\frac{N}{10}$ HCl
or an alkalinity of − 60

Filtrate of culture 3 weeks old: 20 cc. = 16 cc. $\frac{N}{10}$ HCl
or an alkalinity of − 80

The Richards' solution had, in the pure sterile state, an acid reaction:

20 cc. of Richards' solution = 4.8 cc. $\frac{N}{10}$ NaOH or an acidity of + 24.

From this it can be easily seen that the growth of the fungus rendered the medium alkaline, the degree of which increased with the age of the culture, as follows:—

Alkalinity due to one week's growth:	24 + 15	or	39
" " two " "	24 + 60	or	84
" " three " "	24 + 80	or	104

[1] The plus and minus on Fuller's scale denote respectively acidity and alkalinity of the substance.

The effect of the alkalinity of the filtrate on wilting. It was desirable to find out whether the alkalinity of the filtrate was the cause of the wilting.

Solutions of −15, −60 and −80 NaOH and NH_4OH (spec. gr. .88) were made up and compared with the filtrates of cultures one week, two weeks and three weeks old respectively as to their wilting power on broad beans.

The results were as follows:—

Solution	*Time for the first signs of wilting to appear*	
−15 NaOH	5.5	days
$-NH_4OH$	5	"
Filtrate of culture 1 week old having alkalinity of −15	18.5	hours
−60 NaOH	4.5	days
−60 NH_4OH	4	"
Filtrate of culture 2 weeks old having alkalinity of −60	4.5	hours
−80 NaOH	3	days
−80 NH_4OH	3	"
Filtrate of culture 3 weeks old having alkalinity of −80	2.5	hours

The wilting in the different alkaline solutions, namely the NaOH and NH_4OH, was uniform in all the leaves when it became apparent. The cut ends of the bean stems were black in colour in the stronger solutions: namely the −60 and the −80 NaOH and NH_4OH at the time when the wilting became apparent.

It was obvious from the preceding experiment that the alkalinity of the filtrate was not responsible for the wilting produced; moreover when filtrates of cultures from one to three weeks old were either neutralized or rendered acid to the same degree as the original medium, namely the +24, wilting occurred as in the untreated filtrate. Therefore it can be said that though an alkaline reaction develops progressively with the age of the culture, this reaction is not directly responsible for the wilting of the bean stems.

Volatile substances in filtrate. Known amounts of filtrates of different ages were placed in an oven at 100° C. for an hour to allow any volatile substance present in the filtrate to escape. When cooled down to the same temperature as the unheated, distilled water was added to replace that which was lost due to the heating. These were compared with the unheated filtrate of the same age, but showed no loss of their wilting power. It can be said that the factor responsible for the wilting is not a volatile substance.

An oxalate present in the filtrate. Filtrates of three weeks old cultures were tested for an oxalate by the following methods:—

1)—A portion of the filtrate was heated with concentrated sulphuric acid, CO_2 was given off and its presence was detected by its action on lime water.

2)—Also, a portion of the filtrate was neutralized and a solution of calcium chloride was added to it; a white precipitate of calcium oxalate was formed which was soluble in dilute hydrochloric and nitric acids but insoluble in acetic acid.

3)—Also an acid solution of potassium permanganate was added drop by drop to a portion of the filtrate; the colour of the permanganate was discharged, owing to the reduction by the oxalic acid. It is clear that an oxalate is present in the filtrate.

Estimation of the acid radical C_2O_4 in the filtrate of a three weeks old culture: To 50 cc. of the filtrate, a solution of $CaCl_2$ was added, and the precipitate formed was dissolved in dilute HCl and made up to 50 cc.

When titrated with $KMnO_4$ it gave the following average result:—

20 cc. ($\frac{N}{10}$) of filtrate = 4.5 cc. ($\frac{N}{10}$) $KMnO_4$

1 cc. ($\frac{N}{10}$) C_2O_4 " = 1 cc. ($\frac{N}{10}$) $KMnO_4$

1 cc. ($\frac{N}{10}$) C_2O_4 contains $\frac{(12 \times 2) + (16 \times 4)}{2 \times 10 \times 1000}$ or .0044 grm. of C_2O_4, i. e., 4.5 cc.

($\frac{N}{10}$) K M n O_4 = .0044 × 4.5 or .0198 grm. of C_2O_4 or 20 cc. of filtrate contains .0198 grm. ofC_2O_4; ∴ percentage is .099 of C_2O_4 in filtrate.

The wilting power of different solutions of C_2O_4. Solution of (NH4) C_2O_4 containing .099 per cent C_2O_4 or of a strength of $\frac{N}{40}$ C_2O_4 in a litre of distilled water; this solution failed to produce wilting when freshly cut bean stems were placed in it. Even after three days the bean stems were still fresh and healthy.

Similarly a solution of $\frac{N}{10}$ (NH4)2 C_2O_4 was tried but did not produce wilting except after three days, while a solution of $\frac{N}{1}$ produced wilting in three hours. In the $\frac{N}{1}$ solution 4.4 per cent of C_2O_4 was present; while the filtrate of a three weeks old culture, which produced wilting in about the same time as the $\frac{N}{1}$ solution, contained only .099 per cent of C_2O_4.

Though the wilting in both was similar in appearance, it cannot be said that the oxalate present in the filtrate is directly responsible for the wilting.

CONCLUSIONS

(1) *Fusarium Solani*, when grown under artificial conditions, produces a toxic diffusible substance which increases in concentration with the age of the culture. This toxic substance produces a rapid wilting of cut bean stems when these are placed in the filtrate of the fungus.

(2) The culture becomes alkaline due to the growth of the fungus. The degree of alkalinity increases with the age of the culture; this alkalinity, however, does not seem responsible for the wilting.

(3) Ammonia and oxalate are produced by the fungus during its growth, but neither is present in sufficient quantity to explain the toxic action of the solution.

(4) The unknown substance responsible for the wilting is thermostable and non-volatile.

Since the above was written, a paper by Mr. Royal J. Haskel on "Fusarium wilt of potato in Hudson River Valley, New York," has come to my notice (Phytopathology **9**: 223–260, 1919).

This writer describes certain experiments, which lead him to the conclusion that the wilting effect is not due to mechanical stoppage of the water-conducting vessels, but can be explained partly by the introduction of toxic substances into the transpiration current. His experiments suggest that these toxic substances may arise as decomposition products of the host tissue, as well as perhaps by actual secretion on the part of the fungus.

AN EFFECTIVE METHOD OF INOCULATING BARLEY WITH COVERED SMUT

W. H. TISDALE[1]

The uncertainty of infection of barley by covered smut, *Ustilago hordei* (Pers.) Kell. and Sw., when the seed is smutted artificially has caused considerable concern among those investigating varietal resistance of barleys to this disease. Jensen[2] recognized this difficulty of infection in oats and barley as early as 1877 while attempting inoculations in his study of the specific differences between the smuts of these grains. He made the following statement:

"Since we have seen that spores simply adhering to the exterior of the grain do not to any appreciable extent cause infection of the crop it follows that this must take place by means of those spores which succeed in entering the space between the 'cosh' or husk and the kernel."

Jensen believed that infection in oats and barley came from spores which had lodged between the glumes at flowering time. The specific difference of the smuts of these grains, however, had not been determined definitely at that time and some doubt remained as to which of the two smuts of barley Jensen was using. In 1887, he removed the hulls (glumes) from some oat seed and dusted oat smut spores on the seed before sowing. Of the 33 heads produced, four were smutty, while no smut occurred either in plants from seed inoculated with the hulls on or from hulled or dehulled seed uninoculated.[3] In 1888, in a more comprehensive experiment, Jensen found that oat seed, when dehulled and inoculated, produced 21 per cent of smutty heads, while dehulled seed, uninoculated, produced smut-free plants. Dehulled and inoculated barley seed produced plants with 27 per cent of smutty heads, while plants from dehulled seed uninoculated were smut-free. He dipped oat seed in spore-charged water and the plants grown from this seed produced 29 per cent of smutted heads, while seed dusted with spores produced smut-free plants. It is not known which smut of oats Jensen used in his oat experiments.

It is generally reported that naked (hull-less) barley and oats show more smut than do the varieties with the glumes closely adhering to the

[1] The writer is indebted to Mr. J. W. Taylor of the Office of Cereal Investigations, who furnished seed for these investigations.

[2] Jensen, J. L. The propagation and prevention of smut in oats and barley. Jour. Roy. Agric. Soc. **24**: 397–415. 1888.

[3] In this paper, "hulled seeds" are those in the natural state, with the hulls present; "dehulled seeds" are those with the hulls removed.

seed. In view of this fact and the results obtained by Jensen an attempt was made to determine by experiment the importance of the glumes in preventing infection by spores dusted on the hulled seed. In January, 1921, seed of a number of varieties of barley was sown in the greenhouse after dusting thoroughly with spores of covered smut. The resulting plants were free of smut. On November 1, 1921, seed of Tennessee Winter barley from a field practically free from covered smut was dehulled and inoculated and sown along with hulled seed which was similarly inoculated. The hulls were removed by breaking the basal or rachis end of the glumes from over the embryo and removing the remaining parts with a knife or scalpel. The results of this experiment are shown in table 1.

TABLE 1—*Infection, by covered smut, of Tennessee Winter barley grown from hulled and dehulled seed which was dusted with smut spores and sown in the greenhouse at Arlington Experiment Farm, Rosslyn, Va., on Nov. 1, 1921.*

Treatment	Number of heads	Smutted heads	Per cent smutted
Dehulled seed; inoculated	461	392	85.03
Hulled seed; inoculated	403	16	3.97
Hulled seed; uninoculated	357	1	.28
Hulled seed; covered with soil mixed with smut spores	376	10	2.66

The data in table 1 show that removing the hulls was very effective in producing infection in this particular case. Covering the hulled seed after sowing with soil heavily charged with spores had about the same effect as dusting the spores on the hulled seed.

In the fall of 1922, seed of 12 varieties of barley was sown in the greenhouse in a similar experiment. The seed was taken from the 1922 crop grown on Arlington Experiment Farm and some of it was known to be infested with covered smut. This smutted seed was used in order that natural inoculation might be compared with artificial inoculation. The results are shown in table 2.

The data in table 2 show that barley is much more subject to infection by covered smut if the hulls are removed before the seed is inoculated. There are some indications that varieties differ in their susceptibility to this smut. Nakano Wase failed to become smutted under any condition. Tennessee Winter grown from seed dehulled and uninoculated showed slightly more infection than plants from hulled seed which

TABLE 2—*Infection of 12 varieties of barley by covered smut after the seed was inoculated in various ways and sown in the greenhouse at Arlington Experiment Farm, Rosslyn, Va., on October 21, 1922*

Variety	C. I. No.	Treatment	Number of plants	Per cent smutted plants	Number of heads	Per cent smutted heads
Alaska	534	Hulled; uninoculated	27	18.52	173	10.98
		Dehulled; inoculated	29	62.07	115	61.74
		Hulled; inoculated	25	12.00	156	13.46
Cartouch	1107	Hulled; uninoculated	22	0	149	0
		Dehulled; inoculated	14	85.71	77	74.02
		Hulled; inoculated	22	4.54	129	.77
Cusado	895	Hulled; uninoculated	27	11.11	121	14.05
		Dehulled; inoculated	26	57.69	141	46.80
		Hulled; inoculated	29	3.45	128	2.34
Greece	221	Hulled; uninoculated	31	0	125	0
		Dehulled; inoculated	24	79.16	90	56.66
		Hulled; inoculated	27	3.70	126	1.58
Han River	206	Hulled; uninoculated	26	0	156	0
		Dehulled; inoculated	26	73.07	169	61.53
		Hulled; inoculated	25	4.00	173	3.46
Nakano Wase	754	Hulled; uninoculated	not counted	0	not counted	0
		Dehulled; inoculated	do	0	do	0
		Hulled; inoculated	do	0	do	0
Orel	351	Hulled; uninoculated	25	0	116	0
		Dehulled; inoculated	27	37.03	107	57.01
		Hulled; inoculated	28	0	215	0
Scottish Pearl	277	Hulled; uninoculated	25	8.00	186	9.14
		Dehulled; inoculated	22	68.18	177	67.89
		Hulled; inoculated	21	4.76	188	.53
Tenkau	646	Hulled; uninoculated	21	14.28	170	5.88
		Dehulled; inoculated	24	62.50	169	66.27
		Hulled; inoculated	23	4.34	175	1.14

Variety	C. I. No.	Treatment	Numbes of plants	Per cent smutted xlants	Number of heads	Per cent smutted heads
Tennessee Winter	257	Hulled; uninoculated	25	8.00	254	1.96
		Dehulled; uninoculated	22	9.09	191	6.80
		Hulled; grown in greenhouse; uninoculated	25	0	209	0
		Hulled seed; grown in greenhouse; flowers in oculated	23	0	197	0
		Dehulled; inoculated	21	76.19	164	51.22
		Hulled; inoculated	24	25.00	190	10.52
		Hulled; covered with spore-charged soil	26	11.54	195	4.10
Texas Winter	554	Hulled; uninoculated	22	40.90	170	26.47
		Dehulled; inoculated	14	92.82	129	90.62
		Hulled; inoculated	23	4.34	173	1.73
Wisconsin Winter	519	Hulled; uninoculated	24	12.50	182	3.84
		Dehulled; inoculated	18	50.00	125	31.20
		Hulled; inoculated	22	4.54	170	.58

were uninoculated. This seems to indicate that the spores which infect are carried between the hulls and the kernel. However, plants grown from seed inoculated by dusting spores between the glumes at flowering time showed no smut. The high temperature existing in the greenhouse where these inoculations were made might have affected the results. Covering the seed with spore-charged soil after sowing failed to increase the percentage of infection materially.

The sowings listed in table 2 were repeated in the field at Arlington Experiment Farm, but no results were obtained, due to almost complete winter-killing of plants from dehulled seed, which was sown very thinly. If a more efficient means of dehulling the seed could be found or if the inoculation could be accomplished in some other way with less trouble the testing of barleys for resistance to covered smut could be accomplished with less delay. Possibly scarifying the seed before smutting would give the same results as the method of dehulling employed. The hulls seem to be the main obstacle in the way of successful inoculation.

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WASHINGTON, D. C.

SOME POSSIBLE RELATIONSHIPS OF THE MOSAIC DISEASES

H. A. Allard

A mass of published material has accumulated dealing with the results of observations and experiments bearing on the mosaic diseases of the potato, tobacco, and likewise of other plants outside of the Solanaceous Family. A great deal has been learned, but much still remains to be learned concerning all these diseases. Now and again it is a good thing to emphasize what we do not know as well as to keep in mind what we really know concerning any problem. Perhaps it would be just as well if we published our lack of knowledge occasionally, for the time completely divorcing ourselves from favored theories and orthodoxies which may have been unduly emphasized in our papers dealing with positive results.

Let us consider some angles of the problems connected with the mosaic diseases of the Solanaceous plants. The following points have not yet been cleared:

(1) The nature of the infective agent has not been determined, whether bacterial, protozoan, etc.

(2) The actual relation of the mosaic disease of potatoes to the mosaic disease of tomatoes and tobacco is not known; whether or not the two are specifically distinct, or simply due to physiological variations in a common infective agent.

(3) It is not known just how the "virus" is carried over the winter in all instances. Affected perennials such as species of Physalis and *Solanum carolinense* are known to be one factor in the case of the mosaic disease of tobacco and tomatoes. A second and perhaps very important reservoir of the virus may be overwintering plant lice or other insects, but this has not been established. If the virus is carried over the winter by insects, there are two possibilities, one the mere mechanical transfer of the virus through infected mouth-parts, etc., of overwintering insects which have fed upon mosaic diseased plants. In the case of certain plant lice which are known to be active carriers of the virus, it is also possible that a more complex relationship exists between the infective agent and the aphid. Several nice points are involved here. It has already been shown that certain species of plant lice when allowed to feed upon mosaic diseased tobacco, tomato, or potato plants are able to transfer the infective agent readily to healthy plants of the same kind. Is it necessary for each generation to pick up the infective agent anew, or is it possible for the viviparous females to transmit the infective agent to their living

progeny? Finally, is it possible for the infective agent causing these diseases to be transmitted to the overwintering eggs produced by the last generation of the season, ultimately to reappear in the stem mothers and successive generations as a source of infection for the new crop of tobacco, tomatoes, and potatoes? If plant lice which appear to be the natural carriers of the virus of the mosaic diseases serve to carry the virus over the winter, some such method must obtain, and it becomes a question of great importance to establish the truth of these complicated relations. Both phases of the question are equally important since in colder climates the link may be dependent upon the overwintering egg alone, or in the warmer portions of the country the link may be dependent upon overwintering viviparous females themselves. The transfer of the "virus" from generation to generation through viviparous females, or through the egg becomes a more involved relation of a more or less symbiotic nature. There is nothing paradoxical in the possibilities of such relations, since it is already established that very complicated relations may obtain between various disease producing organisms and their alternative insect and animal hosts. The South African Coast Fever is one of these in which the Brown Tick (*Eurhipicephalus appendiculatus*) spreads the blood parasite (*Babesia parva*) among cattle and buffaloes. The life history and host relations are briefly these: The ticks lay their eggs in the grass, these hatching in about 28 days into 6-legged larvae which attach themselves to passing cattle, feeding upon their blood. These fall off in three or four days, remaining dormant about 21 days, then moult, giving rise to 8-legged nymphs. These again pass to cattle, sucking their blood for the same length of time as the larvae, finally dropping off replete, moulting in about 18 days and becoming adult male or female ticks. These attack cattle, mating takes place and egg-laying in the grass completes the cycle of the tick carrying the protozoan parasite. Strange as it may seem only the nymphs in this cycle transmit the disease-producing parasite (*Babesia parva*) to healthy animals although the larvae and adults themselves suck blood as well. Moreover, the larvae, which do not themselves transfer the disease, yet retain the infective agent which appears in an actively infecting form in the nymphs themselves. Here, if the facts are correctly worked out, we have an intermediate stage alone in the life cycle of the tick-host capable of transferring the disease, with blood-sucking stages on either side, the larvae on one, the adults on the other, neither of which transfers infection.

While it has been established without much question that the mosaic "virus" may overwinter in certain perennial plants in some instances,

this does not always appear to account for the sudden appearance and spread of the disease in all localities. The missing link that completes the story may well be an overwintering of the "virus" in the bodies or in the eggs of certain plant lice which are active carriers of infection. The problem is an interesting and important one and may clear up the question of the overwintering of some reservoir of infection at the present time not clearly understood.

Theories in themselves are of no value since they are but tentative explanations of conditions and relations not completely understood. Theories, nevertheless, have their place, their rôle, since they may serve as a vantage point from which to lead out in a problem where real work is being followed. A theory at best is a temporary attitude of the mind and new facts may support or destroy it. A theory becomes old and useless when experience has progressed beyond its conditions and limitations. In any problem involving such intricacies of relationship as the mosaic diseases which include such possibilities as an infectious "virus" carried by particular insects, particular species of plant lice, overwintering in affected perennials, and perhaps in the insect carriers themselves or their eggs, other possibilities of the problem should not be slighted or ignored. The forces of life have developed many strange and incomprehensible relationships as exemplified in the living relationships of hosts of organisms. There is one question that has come to my mind on several occasions which may at first seem fanciful, yet nevertheless in it rests a remote possibility, and no possibility is so remote that it can not bear some relationship to reality. Many fungus and bacterial diseases affect plants, and tobacco, tomatoes, and potatoes are no exceptions. Is it possible for the bacterial cells or the spores or hyphae of invading fungi to become contaminated with the "virus" or actually to absorb or include the infective principle in mosaic-diseased tissues, thus transferring infection in some instances or even serving as an overwintering host? The fact that certain species of fungi and bacteria are so nicely related physiologically to certain host plants as to find their nutrition solely within the sap, tissues, and protoplasm of the latter would suggest that relationship might even exist between some of the "virus" diseases and these more evident, more visible parasites, both of which have become more or less highly adapted to the same physiological environment. This is merely a suggestion, but while we are looking so strenuously for an overwintering reservoir for the "virus" in some of the higher plants or insects, it may be just as logical to eliminate some of the possibilities lower down in the scale among invading bacterial and fungus parasites which may at times become intimately associated with the "virus."

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A METHOD OF ISOLATING SELECTED SINGLE SPORES

JOHN W. ROBERTS

WITH ONE FIGURE IN THE TEXT

In connection with a series of experiments involving spore-selection, it became necessary to isolate not only single spores but also certain individual spores selected from a large number. After selection and isolation the spores were transferred to artificial media for the purpose of permitting study of the resulting growth and further selection from another generation.

The method used was a modification of that employed by Edgerton.[1] The apparatus designed by Edgerton consisted of a capillary tube fastened in an ordinary rubber-tube clamp mounted on a cork which was fitted into the diaphragm opening of the subtage condenser of a microscope in the "swung out" position. Orientation of the open end of the capillary tube, which was simply a tube of a very small bore drawn to a capillary point at one end and closed at the other, was accomplished by turning the whole apparatus and by raising and lowering it by means of the screw which raises and lowers the condenser. The spore and a drop of the water surrounding it were drawn into the tube by applying ether to the closed end and expelled by warming the closed end. The spore was usually forced out upon a sterile cover slip, examined under the microscope and if satisfactory the slip with the drop containing the spore was pressed into media contained in a Petri dish. Observation of the spore was also made during germination to make sure that only one had been picked up.

As the rubber-tube clamp with its elastic-band fastening used by Edgerton to hold the capillary tube was not sufficiently sturdy to give the rigid support desired, a holder was devised to meet this need. A support (Fig. 1, a) commonly found on ring stands and used to hold test tubes or flasks was unscrewed from the clamp holding it to the upright of the ring stand and mounted on the cork by simply twisting the threaded end through the center of the cork. The jaws of the support were lined with cork (b) so that when closed they fitted tightly together save for a groove into which the capillary tube (c) was fitted.

When using the apparatus, the capillary tube (c) is first adjusted so that when swung into position beneath the objective of the microscope, the open end is seen near the center of the field. By turning the entire apparatus the tube is then swung to one side and adjusted to a height

[1] Edgerton, C. W. Phytopath. **4**: 115–117. 1914. 1 fig.

such that when swung back into position it clears the surface of the stage by several millimeters. The slide with a comparatively small number of spores mounted in water is now placed in position and the spore having the desired characters is selected and placed near the center of the field by manipulating the slide. The apparatus is then swung about so that the open end of the capillary tube appears in the

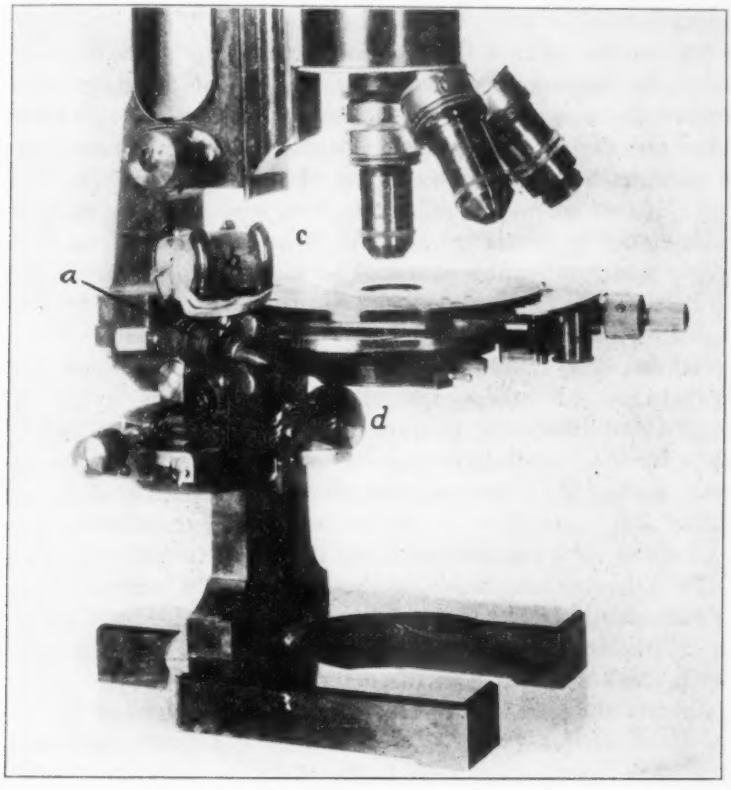

FIG. 1. Apparatus for picking up single spores.

center of the field almost directly above the spore. By means of the lowering screw (d) the end is then lowered until it strikes the water. Sometimes the spore is sucked up immediately but more often it is necessary to increase the suction by applying ether to the closed end of the tube. After the spore in a small amount of water has been taken up, the capillary tube is raised from the water by means of the screw and the whole apparatus is swung to the side. After careful removal of the capillary tube from its support, the closed end is held over a low flame and the open end over artificial media contained in a Petri dish.

By this means the drop of water containing the spore is driven out of the tube and is made to fall into the Petri dish within the circumference of a circle about 3 mm. in diameter previously indicated by marks on the bottom of the dish. As many as a dozen such transfers may be made to one Petri dish. The flame used should be a very small one, else the water in the tube may become too hot or may be changed to vapor. A flame of the small burner known as the "Micro Burner" has been very satisfactory.

As the circles indicate the location of each drop, the Petri dish is placed under the microscope and the contents of the drops examined to be sure that not more than one spore was picked up and to be sure that the one picked up is the one wanted. Another examination is made after germination has begun, at which time every spore is easily located. If two spores should be found in a single drop, growth from that drop may be discarded. Often, however, they are so far apart that their positions can be indicated by marks made on the under side of the Petri dish. The desired spore can then be transferred to another Petri dish or tube of media.

Special attention should be paid to Edgerton's instructions regarding capillary tubes. He advises that they be provided in advance, placed in plugged test tubes and sterilized. He also states that they should be made from as small glass tubes as possible, not larger than 2 mm. diameter inside. In a smaller tube there is less volume of air and the expansion and contraction of this is less, making manipulation easier. Also a smaller tube can be drawn out more satisfactorily than a larger one. To Edgerton's remarks, it should be added that the open end of the tube should be but little wider than is necessary to admit a single spore. With large spores it is possible to have the opening too small, but with smaller spores it is often too large.

To obviate the necessity of alternately turning out and lighting the flame, the ether is kept in a small-mouthed bottle, in the stopper of which a small glass rod is inserted. By this arrangement a drop may be placed upon the closed end of the tube with the release of a minimum quantity of ether fumes into the room.

The method here given may, at times, seem laborious and unduly tedious but by its use one can be absolutely certain that one has isolated not merely a single spore but a particular single spore possessing those characters which he wishes to study. The rigidity of the apparatus here described, permitting of delicacy and accuracy of operation, greatly facilitates the task.

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PHYTOPATHOLOGICAL NOTES

Foot-rot or Ophiobolus in California.—For three or four years the search for foot-rot (*Ophiobolus graminis*) in the grain fields of California failed to positively identify this disease. However, during the past season there appeared in the plots of wheat at Davis plants which appeared to be attacked by Ophiobolus. These plots were irrigated every week during the last four or five weeks of the growing season in order to induce stem rust attack. As the plants came into head the spikes appeared light in color and weight and did not bend under the weight of the filling head. Hormodendron lesions appeared on the glumes as small black blotches. The kernels when examined were found in every case to be badly shriveled and useless for milling or seeding purposes. The plants when pulled came out of the soil readily. The roots were decidedly gray in color and had broken off short where rotting had set in. Enlargement of the roots of diseased plants was common. Such enlarged roots (almost three or four times the normal diameter) when cross-sectioned were found to be hollow throughout the length of the enlarged portion.

The culms were usually weak and looked like those of prematurely ripening plants. In number the culms were not reduced below that for disease-free plants.

Upon the surface of the culm on the first and second internode, usually below the surface of the soil, appeared blackened areas, with lighter areas between. Upon the inner surface of the enveloping leaf sheath appeared black bodies which on examination proved to be perithecia filled with asci containing eight ascospores. The spores were extremely long and linear in shape. The disease was definitely identified as *Ophiobolus graminis* by Dr. H. B. Humphrey.

Specimens collected by the author for the past four years over California indicate that the disease is widespread and apparently has been established in the State for many years and does not appear to be increasing. Foot-rot has been found to cause blighted heads in wheat fields to the extent of 15 per cent of the crop.

Hitherto the specimens observed have failed to show perithecia which made certain identification impossible. The blackened area appeared on the surface of the internode as usual and the mycelial weft or blackened area extended to the inner surface of the enclosing sheath, but no perithecia appeared.

It is believed that the application of irrigation water late in the season gave the Ophiobolus the proper conditions required for the development of perithecia.

At Davis, Ophiobolus perithecia were this year found on barley. Mr. McKinney in 1922 reports that he found barley attacked west of Riverside in Southern California.

So far as the observations have extended no variety of wheat has been

found immune from attack, but certain varieties like Hard and White Federation appear peculiarly susceptible.—W. W. MACKIE.

A new disease of cultivated barley in California caused by Helminthosporium californicum, n. sp.—An undescribed species of Helminthosporium has been observed to be generally distributed and abundant in California on cultivated barley. Its period of development begins much later than that of other Helminthosporium species previously described. It appears with the warm, dry weather and develops rapidly. The apical ends of the leaves are first attacked and finally a "rusty blotch" is formed on the leaves. The lower leaves are first attacked and the disease progresses to the top of the plant. The grain is shriveled due to the premature death of the leaves. The spots are irregular, brown to bluish black, usually merging into diffused brownish discoloration of larger part of leaf. The spots are not netted (*H. teres*), elongated (*H. gramineum*), or definitely bounded dead areas (*H. sativum*).

The conidia of **H. californicum** n. sp., are dark olivaceous when fully mature; fusiform, not curved; thick walled; 40–85 μ × 20–26 μ; average 58 × 22 μ; 3–9 septate. Germinate by single germ tube from any segment.

Resembles *H. sativum* P. K. & B., except that it occurs later, is more severe, the diseased areas are not definite and have no distinct margins. The conidia are longer, wider, more dense, thicker walled and not curved.

The rusty blotch which has been observed for four years in California has been determined to be a new species of Helminthosporium and has been named *H. californicum*. It has been increasing in severity in the Sacramento and San Joaquin Valleys. Studies carried on show marked varietal resistance to this disease.—W. W. MACKIE AND G. E. PAXTON.

Personals—Dr. George H. Pethybridge, formerly Head of the Seeds and Plant Diseases Division of the Department of Agriculture and Technical Instruction for Ireland, has been appointed Mycologist to the Ministry of Agriculture and Fisheries for England. His headquarters will be at Harpenden.

Dr. Paul A. Murphy has been appointed Head of the Plant Diseases Division of the Department of Agriculture and Technical Instruction for Ireland. Dr. Murphy, whose address is Plant Diseases Division, College of Science, Dublin, writes to say that he wishes to keep up close coöperation with his American and Canadian colleagues and would be glad if individuals and institutions which formerly supplied publications of a pathological nature to the Seeds and Plant Diseases Division would send them personally to him.

The November number of Phytopathology was issued November 9, 1923.

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